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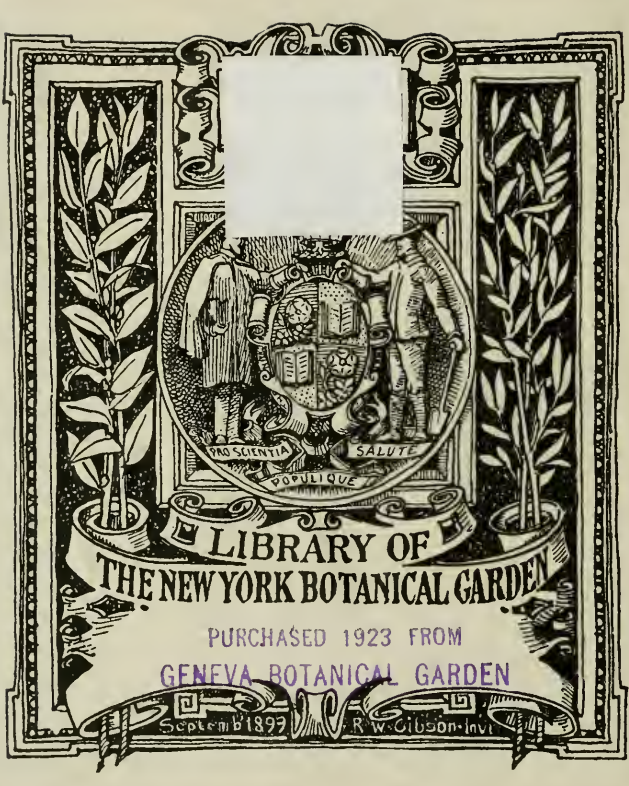
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EDITOR
JOHN MERLE COULTER

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P. 468, legend fig. 1, for 9, *Abies canadensis* read 9, *Abies balsamea*

P. 469, line 10 from bottom, for *Abies canadensis* read *Abies balsamea*

P. 524, line 7 from bottom, for SCHIMPER read SCHIFFNER

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P. 82, line 18 from top, for contact read contrast

P. 83, line 4 from bottom, for successfully read successively

P. 149, line 14 from bottom, for 20 μ read 2.0 μ

P. 160, line 20 from top, for XVI read XVII

P. 164, citation 4, for 1891 read 1892

P. 186, line 6 from bottom, for brought read drought

P. 216, last word line 1, for *virginiana* read *virginica*

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Editor: JOHN M. COULTER

JANUARY 1919

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JOHN M. COULTER

With the assistance of other members of the botanical staff of the University of Chicago

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JANUARY 1919

OENOTHERA RUBRINERVIS, A HALF MUTANT

HUGO DEVRIES

In the spring of 1913 in a culture of *Oenothera rubrinervis* I noticed some young plants, the leaves of which were a little broader than those of the other rosettes. Although the difference was very small, I planted them separately and saw that the deviation did not increase until the time of flowering. The spikes, however, gave proof that the aberrant specimens constituted a type of their own, since the bracts repeated the marks of the primordial leaves, being broader and more flattened than in ordinary *rubrinervis*. There were 7 specimens of the new form among a culture of 25 plants, all of which flowered in August. This indicates a percentage of about 30. In the following year the seeds of the new form gave a uniform progeny, whereas those of the normal specimens repeated the splitting. Thereupon I studied their seeds and found that about one-fourth of those of *O. rubrinervis* were empty, but almost every seed of the new type contained a living embryo. On account of this very small but constant difference the new form was designated as mut. *deserens*.[1] Evidently it might have escaped observation in previous years, the individuals simply being taken for weaker specimens of the type. I studied the progeny of as many self-fertilized specimens of *O. rubrinervis* as were available, therefore, and found the new type among all of them, and as a rule in correspondingly high numbers. Different strains of *rubrinervis* yielded the same result.

[1] Zeitschr. f. Ind. Abst. 16: 262. 1916.

AUG 7 - 1923

If we should apply the principle of BARTLETT concerning mass mutation, and that of MORGAN concerning lethal factors to this case, as I have made use of them in explaining the secondary mutability of *O. grandiflora* and *O. Lamarckiana*,[2] we would conclude that *O. deserens* is a mass mutation of *O. rubrinervis*, and as such is a repetition of the initial mutation which produced the *O. rubrinervis* from *O. Lamarckiana* in my garden. This initial mutation must have occurred in a sexual cell, which, after copulation with a normal gamete of *O. Lamarckiana*, gave rise to a half mutant, *O. rubrinervis*. In other words, *O. rubrinervis* arose as a half mutant between potential *O. deserens* and normal *O. Lamarckiana*. This half mutant, after artificial self-fertilization, must have produced a splitting into three types, exactly in the same way as this splitting can be observed in the half mutants of *O. gigas nanella*. Of these types two must be constant, but the third must repeat the splitting. *O. deserens* is one of the constant ones, whereas the other is assumed to be hidden in the empty seeds, containing a lethal factor just as in *O. grandiflora* and *O. Lamarckiana*. The third type is the continuance of *O. rubrinervis*, and repeats the splitting in every generation.

According to my view *O. Lamarckiana* produces yearly two kinds of gametes in consequence of a secondary mutability into *velutina*. These *velutina* are linked to a lethal factor, which kills them in the young seeds. If we assume that the mutation into *deserens* took place in the typical gametes, leaving the *velutina* unchanged, we would conclude that *O. rubrinervis* consists of two types of gametes, even as *O. Lamarckiana*, but that both of them are in a mutated condition. One is the new *deserens*, without lethal factor; the other is the old *velutina*, linked to a lethal factor. The result of self-fertilization is now easily explained; the copulation of *deserens* gametes among themselves must produce this form, that of *velutina* must give empty seeds, and the combination of the two types must repeat the *rubrinervis* with its splitting capacity.

On the same basis the occurrence of twin hybrids may be explained, the *deserens* gametes giving the *laeta* hybrids; but here we have a considerable advantage over other instances of twin

[2] Mass mutation and twin hybrids of *Oenothera grandiflora* Ait. BOT. GAZ. 65: 377–422. 1918.

hybrids, since both the constituents are available in pure condition for controlling crosses. Any cross which gives twins with *rubrinervis* may be repeated with *O. deserens* and with my *O.* mut. *velutina* (*O. blandina*). In the first case the result must be hybrids of the type *laeta*, in the second case hybrids of the form *velutina*, and the addition of these must simply duplicate the split progeny of the corresponding cross of *O. rubrinervis*. I have made these crosses in a number of cases and found this deduction verified.

Apart from the described secondary mutability into viable *deserens* and dead *velutina* germs, *O. rubrinervis* is not known to possess any noticeable degree of mutability; it has, especially, never produced those mutants which are of so common occurrence in allied mutating forms. Thus we see that secondary mutability is not, in itself, to be considered as a cause of further mutations, and this seems to me to be a fact of paramount interest in the discussion concerning the probable causes of this phenomenon.

The details of the following experiments will give proof of the proposed conception. I shall first give those relating to self-fertilizations and afterward deal with the crosses.

Oenothera rubrinervis originated in my garden from *O. Lamarckiana* quite regularly in a percentage of about 0.1. Every time the visible characters were exactly the same. Between 1890 and 1900 the mutation was repeated 66 times among 66,000 plants.[3] In 1905 I introduced new rosettes of *O. Lamarckiana* from the original locality near Hilversum into my garden, and among their offspring I observed also repeated mutations into *rubrinervis*. The characters were always the same, namely, a pale reddish tinge, narrow and longitudinally folded leaves, a hairy epidermis, cup-shaped flowers, but above all the brittleness of the stems, branches, and petioles, due to the incomplete development of the cell walls in the fibers of the bark and wood. Until now I cultivated mainly two strains, derived from two different mutants of 1895. One of them has given the material for all my crosses, and I shall designate it as the main line. The second line of 1895 was originally destined for control experiments only, but in 1913 it produced the first observed

[3] The mutation theory, English ed. 1:331. 1909.

case of *O. deserens*, as previously mentioned, and since then it has been studied carefully in this respect.

After repeated cultures of pure *O. deserens* had been made and compared with *O. rubrinervis*, the characteristic marks of the two forms became quite clear and reliable, although very small. In mixed cultures the types may even be separated when very young, but some dubious specimens may remain. At the time of flowering these have almost always been shown to belong to the new type. Among the very young rosettes, with only 3–5 leaves, those of *deserens* are broader and more flattened and of a deeper and purer green, resembling therein young plants of *O. Lamarckiana*. These differences increase slowly until the time when the rosettes must be planted out from the boxes into the garden, about the middle of April. The leaves of *O. deserens* have now a broader base and a less pointed top than those of *O. rubrinervis*, besides the marks already given. In July the differences remain very small, the two types reaching the same height at the same period, but the *rubrinervis* begin to flower one or two weeks earlier than the *deserens*. Seen from above, the spikes show narrow, folded bracts in the first type and flat, broad ones in the second type. This character is easily appreciated and wholly reliable; no dubious cases trouble the counting in mixed cultures. The beginning of August, when the *deserens* have opened only a few of their first flowers, is the best time to separate them. A difference in the color has now become clear, the red tinge of the parent type failing in the *deserens*. Here the leaves, bracts, and flower buds are green, and the flowers are also of a purer yellow. A number of smaller marks, which are helpful in the distinction, almost escape description, as, for example, the form of the flowers, their grouping at the top of the spike, and the more erect position of the buds before opening.

The main character of *O. rubrinervis* is the brittleness of all its parts, as already mentioned. It is exactly the same in *O. deserens*. I have broken the stems of all the plants to be mentioned in this article at the time of sorting them out in August or after harvesting their seeds, but no exception has been found to this rule.[4] Hybrids

[4] I have, moreover, injected all the seeds for the sowings of the later years under a pressure of 8 atmospheres during 48 hours, this being the only reliable means of making the germination as complete as possible.

which possess the other characters of *rubrinervis*, but lack the brittleness, are easily recognizable as rosettes of radical leaves as well as during the growth of the stems. They will be designated as *subrobusta*.[5]

The determination of the percentage of mutants in self-fertilized seeds was made in 1916 in the following manner. The specimens were counted in the boxes at the time of planting out in April and the most undoubtful specimens of *rubrinervis* were counted and destroyed. All the others were planted out and tried at the time of flowering in August. By this means the space required for the cultures was reduced to about one-half of what would have been necessary if all the plants had been set out. Some losses were unavoidable and the percentage figures may be a little too small in ordinary cases. Only under favorable conditions do they come up to the amount of the theoretical expectation, namely, one-third of all the individuals. Since my question, however, was mainly to decide whether all specimens of *rubrinervis* split into this form and *deserens*, or whether there are also plants with a uniform progeny, I shall give the figures as I found them.

PERCENTAGE OF *O. deserens* AMONG CULTURES OF *O. rubrinervis*

Seeds of		Parent number	Number of specimens	Percentage of *deserens*	Mean
Third generation in	1910....	1	60	10	
	1913....	2	59	20	
	1913....	3	60	22	
	1914....	4	59	19	19
	1914....	5	60	25	
	1914....	6	59	19	
	1914....	7	60	15	
Fourth generation in	1915....	1	89	16	
	1915....	2	60	25	18
	1915....	3	79	13	
	1915....	4	89	19	

In the first place, I shall now describe the main line of *O. rubrinervis*. Part of the seeds of the original mutant of 1895 had been preserved until 1905; they germinated sufficiently and gave the second generation. From this a third generation was derived in 1910, 1913, and 1914, and a fourth in 1913, 1914, and 1915. All the parents of these generations have been artificially fertilized by

[5] Gruppenweise Artbildung, p. 143. 1913.

myself. In 1914 I observed for the first time a specimen of *deserens* among these cultures, and this in a third generation. Thereupon I sowed in 1916 the self-fertilized seeds of 11 specimens in order to decide whether all of them would repeat the splitting and to determine roughly the percentage of the new type. The results are given on page 5. From these figures we see that all the specimens tried show the same splitting, and that this is always a mass mutation.

The countings for this table were partly made in the stadium of the young rosettes and partly at the time of flowering. In order to prove the correctness of this process, I repeated the sowings in 1917 for those of the parents of which sufficient seed had been preserved, planted out all of their seedlings, and counted them in August, when they were ripening their first fruits. The results are as follows:

Seeds of	Parent number	Number of specimens	Percentage of *deserens*	Mean
Third generation in 1913....	2	48	15	16
1914....	4	55	24	
1914....	6	59	13	
1914....	7	53	13	
Fourth generation in 1915....	1	58	14	
1915....	4	57	19	

Although the cultures were but small, they show that the deviations from the theoretically expected result (25 per cent) do not depend upon the method of counting as used in 1916.

In this race I self-fertilized the first mutant *deserens* observed in 1914 and derived from it a second and a third generation in 1915 and 1916. The second generation consisted of 95 plants, of which 50 flowered; the third was derived from two parents and embraced 77 and 140 specimens, among which 60 and 60 were left to flower. All of these cultures were wholly uniform at the time of planting out as well as during the flowering period. No *rubrinervis* and no new mutants occurred among them. Thus *O. deserens* is seen to constitute a pure and uniform race.

The percentage of empty grains among the seeds has been given elsewhere for this race of *O. rubrinervis*.[6] The determination was made in the harvest of 5 plants of the third generation grown in

[6] Zeitschr. f. Ind. Abst. 16:262. 1916.

1910 and 1915, and in that of two specimens of the fourth generation of 1915. I found 53–68 per cent of germs, with a mean of 60 per cent. Among the specimens of *deserens*, quoted in the same table, 5 belonged to this race; their seeds contained 96, 99, 94, 83, and 58 per cent of good germs. Thus we see that the empty grains, which are a character of *O. rubrinervis*, have disappeared almost wholly in the new mutant.

My second strain of *O. rubrinervis* was derived from another mutant of 1895. It has not been used for any crosses except those mentioned in this article, and which served as control for the experiments in the main line. Part of the seeds of 1895 were sowed in 1907 and yielded a second generation from which a third has been derived in 1913 and a fourth in 1914. I counted the *deserens* for three parent plants as previously described and found the percentages as follows:

PERCENTAGE OF *O. deserens* IN CULTURES OF *O. rubrinervis*

Seeds of	Culture	Number of specimens	Percentage of *deserens*	Mean
Second generation in 1910....	1913	25	28	19
Third generation in 1913....	1914 (A)	70	14	
Third generation in 1913....	1914 (B)	70	16	

The results agree exactly with those deduced from the previous table. The suspicion, however, that in the two last cases the percentage figures were found too low, on account of losses of specimens of *deserens* at the time of planting out, induced me to repeat these sowings in 1916 from preserved seeds, giving them all the care which the previous cultures and the first ones of 1916 had shown to be necessary. Moreover, in 1913 I had self-fertilized a third plant, besides the two mentioned in the table, and also sowed its seeds. In this way I got in 1916 the following percentages for the seeds of the three self-fertilized plants of 1913:

PERCENTAGES OF *O. deserens* IN CULTURES OF *O. rubrinervis*, STRAIN B

Seeds of third generation	Number of specimens	Number of *deserens*	Percentage of *deserens*	Mean
Plant A.....	84	32	38	30
B.....	90	25	28	
C.....	98	22	25	

The result confirms the expectation and shows that the figures given in the former table, although they give proof of the occurrence of mass mutation among the offspring of every plant of *rubrinervis*, are too low for the appreciation of the exact percentage of *deserens*. This must be estimated at about 30 per cent, or almost one-third of the whole progeny. In this race I self-fertilized three mutants in 1913 and two specimens of *deserens* among their offspring in 1915. I cultivated 150+84+180 specimens of the first group and left about one-half of them to flower, and 70+89 plants of the second group, all of which flowered in 1916. I had 573 plants in all, among which 319 bore flowers and fruits. They were all uniformly *deserens*, showing the marks of the type as previously described. No specimens of *rubrinervis* and no new mutants were observed among them. For one mutant of 1913 and for two plants of the second generation in 1915 I determined the amount of germs in the seeds and found 97–96 and 98 per cent, or an almost total absence of empty grains.

Besides the two described families of *O. rubrinervis* I have controlled the seeds of some mutants in order to know whether all of them contained specimens of *O. deserens* and in percentages pointing to mass mutation. I found the following figures:

PERCENTAGE OF *deserens* AMONG THE OFFSPRING OF MUTANTS

Mutant *rubrinervis* from	Number of offspring	Percentage of *deserens*
O. Lamarckiana, 1910.....	25	12
O. Lamarckiana, 1910.....	60	15
O. pallescens, 1911.....	25	16

The strain of *O. Lamarckiana* was derived from a rosette found in the original station near Hilversum in 1905, and the *pallescens* had been a mutant from this same strain.[7] Although the cultures were small, they prove the existence of mass mutation. I sowed the seeds of a specimen of *deserens* from the first culture in 1914, cultivated 25 flowering plants, and found these uniform with the type of their parent.

[7] New dimorphic mutants of the Oenotheras. BOT. GAZ. 62:262. 1916.

Summing up the results of all the tables, we may conclude that all specimens of *O. rubrinervis*, derived from various sources, and the mutants as well as their offspring, show mass mutation into *O. deserens*, besides a considerable number of empty seeds. Taking into consideration the unavoidable losses in the numerical estimations, we may further conclude that *O. rubrinervis* produces about one-fourth empty seeds, and among the living offspring about one-third *O. deserens*, which are constant in their progeny and have no empty grains, or almost none. This points to a relation of 1:2:1 for the whole harvest. The phenomenon is thus shown to be parallel to the splitting of the hybrid mutant of *O. gigas nanella* and to the mass mutability of *O. grandiflora* and of *O. Lamarckiana* itself.

CROSSES BETWEEN O. RUBRINERVIS AND O. DESERENS.—If this explanation is true, it may be confirmed by means of crossing *O. rubrinervis* with its mass mutant. The sexual cells of the first are about one-half *deserens* without a lethal factor, and the rest *velutina* provided with such a factor; those of *O. deserens*, however, are uniformly so. We must expect, therefore, a splitting into almost equal parts of *deserens*×*deserens*=*O. deserens*, and of *velutina*×*deserens*=*O. rubrinervis*. I made both the reciprocal crosses in 1915, cultivated 58 and 50 specimens of their offspring in 1916, and counted them at the beginning of the flowering period in July, finding as follows:

	Percentage of *rubrinervis*	Percentage of *deserens*
O. rubrinervis×O. deserens..	48	52
O. deserens×O. rubrinervis..	78	22

The two types of hybrids resembled their parents exactly, and the figures point to numerical equality of the two groups, although the cultures were only small. Thus we see that the expectation from our formula is confirmed by the experiment.

TWIN HYBRIDS OF O. RUBRINERVIS.—The twin hybrids of *O. Lamarckiana* and *O. grandiflora* are now explained as the result of the mass mutation of these species, but the experimental proof is not complete as yet because neither of these species is known to occur without that form of mutation. In this respect the case of

O. rubrinervis is far stronger, since its two constituents are both represented in my cultures. This fact makes a complete analysis possible, as I have already pointed out. If *O. rubrinervis* is split by some cross into *laeta* and *velutina* on account of its composition of gametes of *deserens* and *velutina*, then the corresponding cross with *O. deserens* must evidently give the same *laeta* and that with *O. Lamarckiana* mut. *velutina* the same *velutina*. Thus the split progeny can be duplicated by the addition of its components.

I have described the splitting crosses in *Gruppenweise Artbildung* (pp. 122, 196–200, 1913) and repeated some of them so as to have the dimorphic progeny together with the cultures of the presumed constituents, in order to be able to identify their characters during the whole time of their development. The percentage figures given in my book are as follows:

TWIN HYBRIDS OF *O. rubrinervis*

Cross	Percentage of *laeta*	Percentage of *velutina*
O. biennis×rubrinervis	30–49	51–70
O. rubrinervis×O. biennis Chicago	39–44	56–61
O. rubrinervis×O. Cockerelli	49	51
Mean	42	58

In the second and third generation of the two latter crosses the *laeta* have split off brittle *rubrinervis* in about one-third of the cultures, whereas the *velutina* remained constant.

I repeated the two first named crosses in 1915, but not the third one. On the other hand, I have repeated the cross with *O. Hookeri*, in the progeny of which I had previously not been able to distinguish the twin types. I had the following cultures in 1916. Most of these plants flowered in August.

TWIN HYBRIDS OF *O. rubrinervis;* CULTURES OF 1916

Cross	Number of specimens	Percentage of *laeta*	Percentage of *velutina*
O. biennis×rubrinervis	59	46	54
O. rubrinervis×O. biennis Chicago	60	53	47
O. Hookeri×rubrinervis	60	20	80
Mean		40	60

The results coincide with the previous ones as nearly as might be expected. The types of the twins were the same as those in the older cultures.

For these twins I have determined the amount of empty grains, self-fertilizing two specimens of each of them in August 1916 and counting out 100 seeds for each parent.

PERCENTAGE OF GERMS IN SEEDS OF *laeta* AND *velutina*

Cross	Parent number	*laeta*	*velutina*
O. biennis×rubrinervis	1	93	3
	2	96	4
O. rubrinervis×Chicago	1	94	28
	2	95	31
O. Hookeri×rubrinervis	1	94	59
	2	95	71

As in other cases, the seeds of the *laeta* hardly contain any empty grains, whereas those of the *velutina* are often badly developed.

My task was now to repeat these crosses, substituting *O.* mut. *deserens* and *O.* mut. *velutina* for *O. rubrinervis*. The latter group of crosses have already been described elsewhere;[8] they yielded pure cultures of *velutina* which in every case were exactly like the *velutina* of the corresponding cross with *O. Lamarckiana*. The crosses with *O. deserens* were made in 1915 and their progeny studied in 1916; it was in every case wholly uniform.

CROSSES OF *O.* MUT. *deserens*

Cross	Number of specimens	Type
O. biennis×deserens	60	O. (bien.×Lam.) laeta
O. syrticola×deserens	70	O. (syrtic.×Lam.) laeta
O. Hookeri×deserens	60	O. (Hook.×Lam.) laeta
O. deserens×biennis Chicago	70	O. (Lam.×Chic.) laeta

About one-half of each culture flowered and developed their fruits. For each culture a control parcel was cultivated with the *laeta* from the corresponding cross with *O. Lamarckiana*, and they were compared during all the time of their development. I could

[8] Kreuzungen von *Oenothera Lamarckiana* mut. *velutina*. Zeitschr. f. Ind. Abst. 17:1917.

not find any differences. The descriptions for the *deserens laeta* are exactly the same as those given previously for the *Lamarckiana laeta*. Although I have not made the cross *O. syrticola*×*O. rubrinervis*, I have added the second experiment of the table. From this and the result of *O. syrticola*×*O. blandina* (mut. *velutina*) described in my former article the result of the cross *O. syrticola*× *rubrinervis* may be predicted, and so it would be in other cases also.

Summing up the results of these experiments, we see that in producing twin hybrids *O. rubrinervis* is split in exactly the same way as an artificial mixture of about equal parts of gametes of *O. deserens* and *O.* mut. *velutina* would be. The conclusion that its gametes really possess this dimorphy is thereby as clearly proven as might be expected.

CROSSES OF O. RUBRINERVIS WITH O. LAMARCKIANA AND ITS DERIVATIVES.—In *Gruppenweise Artbildung* I have described the first generation of these crosses as consisting of two types, *O. Lamarckiana* and *O.* hybr. *subrobusta*. The latter is a *rubrinervis* in which the brittleness fails, and thereby very similar to our new mut. *erythrina*;[9] but this similarity is only an external one, since after self-fertilization the hybrid *subrobusta* splits off, as a rule, brittle *rubrinervis* plants, whereas the *erythrina* produces the *decipiens*, which is not brittle. Shortly after publishing my book, however, I discovered in the summer of 1913, among the progeny of a cross of *O. rubrinervis* and *O. Lamarckiana*, a slight difference among the *Lamarckiana*-like plants. Some of them were stouter and had broader and less crinkled leaves than the others. I self-fertilized them and got a culture, which, although not uniform, repeated the deviating marks of the parental type in the majority of the individuals. I shall call this hybrid type *lucida*. Moreover, in making a large number of crosses of individuals of the same family of *rubrinervis* with *Lamarckiana* plants from various sources, as well as different mutant strains, I discovered that the second hybrid type is not always the solid *subrobusta*, but sometimes the brittle *rubrinervis*. I have not as yet discovered why this should be so. We should expect the brittleness to be recessive to the production of

[9] Zeitschr. f. Ind. Abst. 16:262. 1916.

strong fibers, and as a rule it is so, but not always. The two contrasting cases have occurred mainly in strains derived from different initial plants, and some hidden mutation might be responsible for the dominance of the brittleness. This seems to be the case at least in *O. nanella*, but the number of crosses in each of my different families of dwarfs is too small to decide whether this is the real cause. Crosses of *O. rubrinervis* with other mutants than the dwarfs have also given sometimes the brittle form and sometimes the *subrobusta* for the second hybrid.

If we keep in mind that the hybrid *rubrinervis* is only a brittle form of the hybrid *subrobusta*, and that the one may be substituted for the other for unknown reasons, the following descriptions will easily be understood. I might add, however, that from a single cross between two individual parents both types never arise simultaneously in the first generation. In the succeeding generations the *rubrinervis* as a rule are constant, whereas the *subrobusta* may split off the brittle form.

If we assume the gametes of *O. Lamarckiana* to consist of equal parts of typical ones and of *velutina*, and those of *O. rubrinervis* to consist of *deserens* and *velutina*, *O. Lamarckiana*×*O. rubrinervis* must yield 25 per cent *typica*×*deserens*, 25 per cent *typica*×*velutina*, 25 per cent *velutina*×*deserens*, and 25 per cent *velutina*×*velutina*. The last-combination will produce empty grains, since the same lethal factor comes in from both sides; on the other hand, the three first named combinations must give viable seeds. *Typica*×*velutina* is the formula for *O. Lamarckiana*, and *velutina*×*deserens* that for *O. rubrinervis* and *subrobusta*, and so the occurrence of these hybrid types is easily explained. The remaining combination *typica*× *deserens* must then be assumed to give the new hybrid *lucida*, and this can be verified by crossing *O. deserens* with *O. Lamarckiana*. All these deductions are, of course, the same for the reciprocal crosses. If these deductions are reliable, they show that the polymorphy of the first generation of hybrids between the two older forms is due to the combination of their capacities to produce twins in other crosses. In other words, it is a natural sequence of their secondary mutability. I shall now describe the experiments which seem to me to justify these deductions.

O. LAMARCKIANA×O. RUBRINERVIS.—According to the deductions just given the expectation for this cross is

O. Lamarckiana×O. rubrinervis=
(typica+velutina)×(deserens+velutina)=
typ.×des.+vel.×des.+typ.×vel.+vel.×vel.=
lucida subrobusta Lamarckiana empty grains
or
rubrinervis

In the first place, I determined the amount of empty grains, using the same method as in previous cases.

Cross	Parent number	Cross	Percentage of germs
O. Lamarckiana×rubrinervis.....	1	1913	21
	2		29
	3		33
O. rubrinervis×Lamarckiana.....	1	1913	48
	2		60

The presence of empty grains is thereby proven, although the percentages of germs are much smaller than would be expected; but this may be due to quite different causes, as has been shown elsewhere.

In the second place, I studied the living progeny for the fourth cross of this table and for a cross made in 1907 with another strain of *O. Lamarckiana*. Each of these cultures was trimorphous, containing the types *Lamarckiana* and *lucida*, and besides these either *subrobusta* or *rubrinervis* (that is, tough or brittle).

Cross	Number of Specimens	Percentage of		
		Lamarckiana	*lucida*	*subrobusta* or *rubrinervis*
O. rubrinervis×Lamarckiana 1913	60	32	20	48 rubrinervis
1907	69	40	6	54 subrobusta
Mean.....	.....	36	13	51

The expectation would be for equal parts, but for some unknown reason the *lucida* almost always fall short of this. Apart from this difficulty the results of these cultures coincide with the theoretical

deductions from our formula. I have made quite a number of further crosses between these two forms, partly in 1905 and partly in 1913, using always the same family of *rubrinervis*, and taking the combinations in both reciprocal directions. Six of them have given for the third hybrid type *rubrinervis* and three of them *subrobusta;* but since I have not determined the amount of *lucida* among them, it is of no use to give the percentage figures.

The exactness of the identification of the types in the formula can be controlled by direct crosses with the constituents mut. *deserens* and mut. *velutina*. The latter has been described under the synonym *O. blandina*. I made the following combinations:

Cross	Year	Number of plants	Percentage of		
			lucida	*subrobusta*	*velutina*
O. deserens×Lamarckiana	1915	49	18	82	0
O. rubrinervis×blandina	1915	70	0	50	50
O. blandina×rubrinervis	1913	70	0	53	47
O. deserens×blandina	1915	70	0	100	0
O. blandina×deserens	1915	49	0	100	0

The expectation for these crosses was:

O. deserens×Lamarckiana = O. deserens×(typ.+velutina) = lucida+subrobusta
O. blandina×rubrinervis = O. blandina×(deserens+velutina) = subrobusta+velutina
O. blandina×deserens = O. blandina×(deserens) = subrobusta

Apart from the figure for *lucida*, which is too small, the results of the experiments directly confirm the expectation. I have determined the amount of empty seeds for the four last named crosses, and found almost none:

Cross	Percentage of germs in seeds
O. rubrinervis×blandina	97
O. blandina×rubrinervis	91
O. deserens×blandina	100
O. blandina×deserens	90

Moreover, I made the same determinations for the hybrids from the two first named of these crosses, self-fertilizing them in 1916. For the two latter crosses it was evident that the hybrids would

hardly have any empty grains, and I did not think it necessary to control this.

Cross	Parent number	Percentage of germs in seeds of	
		subrobusta	*velutina*
O. rubrinervis×blandina	1	96	70
	2	97	75
	3	99	76
	4	97	68
O. blandina×rubrinervis	1	96	75
	2	96	80
	3	96	.....

The *laeta* have hardly any empty grains, but the figures for *velutina* fall short of this, even as in other instances. In the last place, I counted the germs in the hybrids of the crosses with *Lamarckiana*, self-fertilizing their specimens of each of the types:

Cross	Parent number	Percentage of germs in seeds of		
		lucida	*Lamarckiana*	*rubrinervis*
O. rubrinervis×Lamarckiana	1	87	25	53
	2	91	34	59
	3	94	93	65
O. deserens×Lamarckiana	1	85	.....	.....
	2	86	.....	.....

The *lucida* have almost no empty grains; the figures for hybr. *rubrinervis* are the same as those for the mutant of that name, but those for the *Lamarckiana* type give an unexpected result. In two cases they are the same as for the species, but in the third the empty grains have almost wholly disappeared. This latter specimen has lost all the external marks of *O. deserens* and *O. rubrinervis*, but kept the absence of the lethal factors. Its progeny splits into *Lamarckiana*, *lucida*, and *rubrinervis*, and the first of these forms repeats the splitting in the following generation.

O. LAMARCKIANA NANELLA×RUBRINERVIS.—As in so many other cases, the crosses with dwarfs can give a verification of those with the species itself. In *Gruppenweise Artbildung* (p. 215) I have

described the pedigrees of two reciprocal crosses, both of which produced as a second hybrid the *subrobusta*. This was seen to split off, after self-fertilization, brittle plants and dwarfs. In 1915 I sowed some seeds of the *subrobusta* plants of 1907 mentioned in those tables, in order to compare their progeny with my newer cultures. I found for two specimens of *O.* (*nanella*×*rubrinervis*) *subrobusta* 38 and 45 per cent of dwarfs among 82 and 60 plants, and for two parents *O.* (*rubrinervis*×*nanella*) *subrobusta* 20 and 13 per cent of dwarfs among 60 and 46 individuals. The number of brittle plants, however, was very small, being two specimens for the first and one for the reciprocal group. It is possible that the germs of this type are weaker, and that some of them had died during the 7 years of their preservation. I self-fertilized one brittle specimen in each of the two main groups and had in 1916 two lots of 45 and 60 flowering plants, all of which were brittle and like their parents. They contained 9 and 8 per cent of dwarfs, the stems of which were likewise brittle at the time of flowering.[10]

Other races of *O. nanella* or other conditions may produce in the corresponding crosses brittle hybrids instead of *subrobusta*. I made the cross *O. rubrinervis*×*nanella* in 1905 with a dwarf mutant race of 1895 and the reciprocal one with the progeny of a dwarf which had arisen in 1911 from *Lamarckiana*, using in both cases the same family of *rubrinervis* as in all previous crosses. The first named cross gave 35 per cent *Lamarckiana*, 3 per cent *lucida*, and 62 per cent brittle *rubrinervis* among 68 specimens in 1913. The second cross yielded the same three types, but the percentage figures deviated widely. I had only 6 per cent *Lamarckiana* and 2 per cent *lucida*, but 92 per cent brittle *rubrinervis* among 140 plants, most of which flowered in August. The main result, however, is clear, namely, that the crosses between *O. rubrinervis* and *O. nanella* give three types of viable hybrids, one of which carries the visible marks of *O. rubrinervis*, but may be either brittle or tough.

I have made only one cross between *O. deserens* and a dwarf, taking this latter from the first of the two last mentioned families.

[10] By means of this the gap left in the second pedigree of p. 215 of my book is filled up, and both pedigrees are completed by the production of dwarfs from the *rubrinervis* specimens.

I crossed them in 1915 and had in 1916 a culture of 60 plants, among which 3 per cent were *lucida* and 97 per cent brittle *rubrinervis*. Other types failed, as was to be expected. The seeds of the two *lucida* plants contained 89 and 95 per cent of good germs.

Summing up the results of the crosses between *O. rubrinervis* and *O. nanella*, we see that they yield exactly the same hybrid types as those with *O. Lamarckiana* and in corresponding percentages.

CROSSES OF O. RUBRINERVIS WITH HETEROGAMIC MUTANTS.— Crosses with the pollen of these forms must simply confirm those with *O. Lamarckiana*, since their pollen carries mainly the same hereditary qualities as that of the parent species. I fertilized in 1913 two plants of my main race of *O. rubrinervis* with *O. cana*, two with the pollen of the *Lamarckiana*-like offspring of self-fertilized *scintillans*, and added the reciprocal cross of the latter combination. In the following table I shall call these offspring *scintillans-Lamarckiana*.

CROSSES OF *O. rubrinervis* WITH HETEROGAMIC MUTANTS

CROSS	NUMBER OF SPECIMENS	PERCENTAGE OF		
		Lamarckiana	*lucida*	*subrobusta*
O. rubrinervis × cana	60	32	38	30
O. rubrinervis × cana	57	53	32	15
O. rubrinervis × scintillans-Lamarckiana	60	13	3	84
O. rubrinervis × scintillans-Lamarckiana	60	7	10	83
O. scintillans-Lamarckiana × rubrinervis	84	7	4	89
O. scintillans-Lamarckiana × rubrinervis	34	15	9	76

About one-half of each group flowered in August. No brittle specimens occurred. The types were exactly the same as those derived from the cross between *O. rubrinervis* and *O. Lamarckiana*.

If the heterogamic types are used as female parents, the splitting of course will be more complicated. I fertilized a strong biennial specimen of *O. scintillans* with the pollen of a plant of *O. rubrinervis* and had in 1916 a culture of only 23 plants, all of which flowered in August. There were 5 types: 11 *Lamarckiana*, 2 *lucida*, 1 *sub-*

robusta, 9 *scintillans*, and 8 *oblonga*. The first three were the same as in previous crosses and confirm their result; the last two named types are the same as are always seen in the first generation of crosses of *O. scintillans* when this is used as the seed parent.

Moreover, in 1915 I fertilized 4 plants of my race of *O. lata* with *O. rubrinervis*, counted the *lata* and *albida* in their progeny in May 1916, and for want of space planted out only a part of the others, in order to distinguish the types, but without trying to determine percentage figures. Altogether I had 434 seedlings, among which 7 per cent were *lata* and 6 per cent *albida*. At the time of flowering I counted 23 *Lamarckiana*, 1 *lucida*, 20 brittle *rubrinervis*, besides 11 mutants (5 *oblonga*, 5 *obovata*, and 1 *scintillans*). No *subrobusta* occurred in these cultures. These results confirm those previously given.

SECOND AND LATER GENERATIONS.—Brittleness and dwarfish stature are recessive characters, and as such may be expected to be split off in the succeeding generations. For the crosses between *O. rubrinervis* and *O. nanella* this splitting has already been dealt with. For the other crosses our analytical formula for *O. Lamarckiana*×*rubrinervis* shows that the types *lucida* and *subrobusta* may be expected to produce a splitting, whereas the *Lamarckiana*-like hybrids cannot contain the necessary factors. The production of brittle plants from *subrobusta* had been observed in the case of the dwarfs, and so I studied in 1916 the progeny of three specimens of *lucida* from previous crosses.

SPLITTING PROGENY OF *O.* HYBR. *lucida;* CULTURES OF 1916

LUCIDA FROM	NUMBER OF SPECIMENS	PERCENTAGE OF	
		Tall plants	*deserens*
O. rubrinervis×scintillans	67	52	48
O. rubrinervis×scintillans	56	80	20
O. rubrinervis×Lamarckiana	106	53	47
Mean		62	38

Moreover, in 1916 I self-fertilized some specimens of *lucida* taken in the first generations of the crosses mentioned, sowed their seeds in 1917, cultivated all the seedlings until the time of ripening their first fruits, and counted them repeatedly during the summer. The

difference between the *deserens* and the *lucida* was very striking, the first reaching only half the height of the latter. I have broken the stems of all the plants in August, at the time of the last counting, and found all the *deserens* brittle and all the tall ones tough. The first were evidently *deserens* and not *rubrinervis*, as seen by the characters described for these two types. Among the tall ones, however, I have not succeeded in finding any difference, the type of *lucida* prevailing to the apparent exclusion of that of *O. Lamarckiana*. For each of the crosses mentioned in the following table I had 58–60 flowering specimens in August.

SPLITTING PROGENY OF HYBRID *lucida;* CULTURE OF 1917

LUCIDA FROM	PARENT NUMBER	PERCENTAGE OF	
		Tall plants	*deserens*
O. rubrinervis×O. Lamarckiana	1	50	50
	2	60	40
	3	48	52
O. deserens×O. Lamarckiana	1	55	45
	2	39	61
O. deserens×O. nanella	1	51	49
	2	41	59
Mean	.....	49	51

Oenothera Lamarckiana mut. oblonga and mut. nanella

Our conception of *Oenothera rubrinervis* as a half mutant may be applied to *O. oblonga*, and explain its behavior in crosses in an analogous way. The main difference, as I have pointed out in *Gruppenweise Artbildung*, is that some types of hybrids, as we might expect, are constantly absent or suppressed, as I called it. If we assume this suppression to take place in the pollen before fecundation, the remaining phenomena are easily explained on this basis. It will be sufficient to review the facts given in my book, and to combine them with the results of some determinations of the amount of barren grains in the seeds of self-fertilized and crossed individuals.

The amount of empty seeds is about the same in *O. oblonga* as in *O. Lamarckiana*. For the cultures of 1911, mentioned in my book, I found among the seeds of two self-fertilized individuals 25 and 33 per cent of germs. Seeds of biennial plants collected in 1913 contained 30–18 and 17 per cent of germs; but seeds of annual

plants, saved in 1914 on two new mutants from *O. Lamarckiana* and on one from *O. cana*, gave only 6–5 and 6 per cent of germs. Annual specimens are always much weaker than biennial ones, and their fruits are often thin instead of club-shaped. These figures evidently point to a complete analogy with *O. Lamarckiana*.

The question whether the lethal factors are the same as in *O. Lamarckiana* may be answered by crosses with this species. I tried the seeds of a cross *O. oblonga*×*Lamarckiana*, of one of *O. oblonga*×*nanella*, both made in 1911, and of a cross of 1913 of *O. oblonga*×*O. cana*. I found 53–40 and 34 per cent of good germs. The figures do not essentially differ from those found for self-fertilized *Lamarckiana*, and thereby show that the lethal factors must be the same and simply inherited by *O. oblonga* from its parent species without change.

The ovules which produce empty grains after self-fertilization may develop into normal seeds after crosses with other species, even as in the case of *O. Lamarckiana* itself. *O. oblonga*×*biennis* gave 92 per cent of germs, *O. oblonga*×*atrovirens* (*cruciata*) 87 per cent, *O. oblonga*×*Hookeri* 90 per cent, and *O. syrticola* (*muricata*)× *oblonga* 90 per cent. Thus we see that in this respect also the lethal factors are the same as in *O. Lamarckiana*.

Our assumption is that *O. oblonga* arises by means of a mutation in the *Lamarckiana* gametes of our species, leaving the *velutina* gametes unchanged. The formula for self-fertilization, assuming the *oblonga* gametes to be suppressed in the pollen before fecundation, is as follows: (obl.+velu.)×velu.=obl.×velu.+velu.×velu. This explains the constancy of the mutant, since the *velutina*× *velutina* germs contain the same lethal factor on both sides and thus produce the empty grains. If we compare this formula with the results of the crosses described in my book (pp. 266–267), we find a complete harmony, as I shall now try to show.

Fertilized by *O. Lamarckiana* and analogous mutants, *O. oblonga* must give (obl.+velu.)×(Lam.+velu.)=obl.×Lam.+obl.×velu. +velu.×Lam.+velu.×velu.=25 per cent empty grains+25 per cent *oblonga*+25 per cent *Lamarckiana*+25 per cent empty grains. The expectation is therefore for two types and these in equal proportions. The two types always appeared, and no others besides

them, but the percentage figures are very variable. I found them as follows:

Cross	Parent number	Percentage of	
		oblonga	*Lamarckiana*
O. oblonga×Lamarckiana	1	4	93
O. oblonga×Lamarckiana	2	4	96
O. oblonga×Lamarckiana	3	14	85
O. oblonga×nanella	1	15	80
O. oblonga×nanella	2	46	14
O. oblonga×scintillans	1	81	17

The reciprocal crosses cannot produce any *oblonga*, since this is assumed to be suppressed in the pollen. The only exception is *O. scintillans*, which gives rise to a high amount of *oblonga* after self-fertilization, and therefore may produce the same mutant after a cross. I found as follows:

Cross	Percentage of	
	oblonga	*Lamarckiana*
O. Lamarckiana×oblonga	0	100
O. nanella×oblonga	0	100
O. lata×oblonga	0	100
O. scintillans×oblonga	18	82

The pollen of *O. oblonga* must produce, after crosses with different species, only *velutina*, as is easily seen from our formula. No *oblonga* and no *laeta* are to be expected. *O. biennis*, *O. syrticola*, *O. Cockerelli*, and *O. Hookeri* fecundated with *O. oblonga* uniformly gave this result. The reciprocal crosses, however, must give a splitting, the *laeta* hybrids assuming the characters of *O. oblonga*. The percentages should be about 50, but in my experiments there was much fluctuation in this respect. I found as follows:

Cross	Parent number	Percentage of	
		oblonga	*velutina*
O. oblonga×biennis Chicago	1	8	92
O. oblonga×biennis Chicago	2	16	84
O. oblonga×biennis Chicago	3	38	62
O. oblonga×Cockerelli	1	41	59
O. oblonga×Hookeri	1	11	89
O. oblonga×Hookeri	2	24	75

Crosses with *O. rubrinervis* also yield the expected result. This would be in one direction (obl.+velu.)×(deserens+velu.)=obl.× des.+obl.×velu.+velu.×deserens+velu.×velu.=25 per cent (*obl.* ×*des.*)+25 per cent *oblonga*+25 per cent *rubrinervis*+25 per cent empty grains. I have not as yet tried the cross between *O. oblonga* and *O. deserens*, however, and thus must leave undecided the question as to which characters will dominate in this hybrid. As a matter of fact, I found 20 per cent *oblonga* and 80 per cent *rubrinervis* and no other types. The reciprocal cross must give (des.+ velu.)×velutina=des.×velu.+velu.×velu.=50 per cent *rubrinervis*+50 per cent empty grains. Only *rubrinervis* have been observed in this culture.

Crosses with the pollen of *O. biennis* must give *oblonga*×*biennis* +*velutina*×*biennis*. The former is intermediate between the parents, whereas the second is the same as the hybrid type *Lamarckiana* ×*biennis*. I found in one cross 65 per cent *oblonga* (partly dwarfish) and 35 per cent hybrids of the second type. In another instance, however, the *oblonga* failed from some unknown reason.

With those species which ordinarily produce the twins *densa* and *laxa* the pollen of *O. oblonga* must evidently give only the latter type. This has been the case in three trials with *O. biennis Chicago* and in one with *O. atrovirens* (*cruciata*).

For further details and for the constancy or splitting in the second generation I must refer the reader to the pages of my book already quoted. These results, however, show clearly that all the facts hitherto ascertained confirm the formula assumed for the self-fertilization, and thereby the analogy with the phenomena observed in *O. rubrinervis*.

Summing up this discussion we may say, therefore, that *O. oblonga* arises through a mutation of the typical sexual cells of *O. Lamarckiana*, leaving the *velutina* gametes and also the lethal factors unchanged, but producing, besides the externally visible marks of the mutant, a suppression of the mutated pollen grains.

On the other hand, *O.* mut. *nanella* seems to arise through mutations in the *velutina* gametes of *O. Lamarckiana*, as is shown by the fact that the *laeta* do not split off dwarfs, whereas the *velutina* regularly do so. The figures given in my book for the crosses with *O. nanella* may be calculated in the same way, and

will be found to comply with the views proposed in this article. It would lead us too far, however, to reproduce these calculations here.

In all these cases the conception that mass mutation is the chief cause of the production of twin hybrids evidently makes the supposition of a labile condition of the factor for *laeta* superfluous. It seems desirable, therefore, to lay stress on the fact that this supposition does not rest on the phenomena observed in the production of these twins. It is mainly derived from other observations, and some of them may be briefly repeated here in order to make this point clear. They refer to the brittleness of *O. rubrinervis* and *O. deserens* and to the dwarfish stature of *O. nanella*.

In crosses brittleness behaves in three different ways. With *O. biennis Chicago* and *O. Cockerelli* it is recessive to the tough structure of the fibers, since it fails in the first generation and reappears in the second in ratios corresponding to Mendel's law. In crosses with *O. Lamarckiana* it is sometimes dominant and sometimes recessive, as has been shown. In *O. rubrinervis* and *O. deserens* the toughness is wholly absent. From these and other facts it is clear that at least three conditions of this factor are possible. I call them active, labile, and inactive. Whether the labile condition is due to linkage or to some other cause is as yet an open question, which, however, has no influence upon the main contention. The combination "active×inactive" is assumed to be responsible for Mendelian crosses, but the combination "labile×inactive" may cause a splitting in the first generation and produces, as a rule, constant hybrids. The two types of first generation hybrids appear in variable numerical proportions according to different circumstances. If one of the groups is so small as not to be represented in every 100 specimens, the splitting may seem to fail, and such extremes are of common occurrence. This would explain the dominance of an evidently recessive character.

The case is exactly the same for the dwarfish stature. The factor for tallness must be in the inactive condition in the dwarfs, but in the active condition in *O. rubrinervis*, since the crosses between these two types follow Mendel's law. In *O. Lamarckiana*,

however, it is labile, since tall and low specimens appear in the first generation of its cross with *O. nanella*. In many ternary crosses of hybrids of this mutant the dwarfish stature dominates over the tall condition, but the dominance is not always absolute and sometimes 3–5 per cent of tall specimens appear among the dwarfs, as I have shown in *Gruppenweise Artbildung*. This fact evidently supports our conception.

The conclusion from this discussion is that since brittleness and dwarfish stature are in some cases recessive to and in other cases dominant over their antagonists, these latter must be sometimes in the active and in other instances in the labile condition.

Summary

1. *Oenothera rubrinervis* is a half mutant, produced by the copulation of a mutated gamete with a normal *velutina* gamete of *O. Lamarckiana*.

2. In consequence, it produces about one-fourth empty grains, a mass mutation of about one-fourth pure or double mutants, and one-half specimens of *O. rubrinervis*, which will repeat the splitting.

3. The pure or double mutant is called *O.* mut. *deserens*. It is very similar to *O. rubrinervis*, but the leaves of its young rosettes and the bracts of its flower spike are broader and more even.

4. *O.* mut. *deserens* is constant from seed. It has no hereditary empty grains.

5. The formula for the self-fertilization of *O. rubrinervis* is therefore *O.* (*deserens*+*velutina*) = *des.*×*des.*+*velu.*×*velu.*+*des.*×*velu.* The first combination gives the mass mutation, the second the empty grains, the third the normal plants of *O. rubrinervis*.

6. In crossing with other species the two kinds of gametes will produce twin hybrids, as, for example, *laeta* and *velutina*. This assertion has been controlled by making the corresponding crosses of *O.* mut. *deserens* and *O.* mut. *velutina*. The first produce the *laeta* and the second the hybrid *velutina*. The result of a cross of *O. rubrinervis* is equal to the sum of these two crosses.

7. Outside of the mass mutability into *O. deserens*, *O. rubrinervis* is not known to mutate to any noticeable degree. This shows that

the internal constitution, which causes the mass mutation, is not in itself a cause for further mutability.

8. The constitution of the gametes of *O. rubrinervis* can directly be proven by a cross with *O. deserens*, since *O. rubrinervis* = (*deserens* + *velutina*) × *O. deserens* produces *O. deserens* and (*O. deserens* × *velutina*) or *rubrinervis*.

9. Crosses of *O. rubrinervis* with *O. Lamarckiana* give three types of hybrids, besides about one-fourth empty seeds. One type exactly resembles *O. Lamarckiana* and is constant in its progeny. A second type called *lucida* has broader and more shiny leaves, and after self-fertilization splits off brittle specimens. The third type is either *subrobusta* or *rubrinervis*, and in the first case may produce the brittle form in the second generation. All these phenomena are easily explained by the proposed formula for the constitution of *O. rubrinervis* as a half mutant. They were confirmed by means of crosses with *O. nanella* and some other mutants.

10. *O. oblonga* is quite analogous to *O. rubrinervis*, since it must arise through a mutation of the typical sexual cells of *O. Lamarckiana*, leaving the *velutina* gametes unchanged. Contrary to *O. rubrinervis*, however, the two lethal factors remain in their condition, and moreover the mutated gametes must be assumed to become suppressed in the pollen of the mutant.

11. *O. nanella* seems to arise through mutations in the *velutina* gametes of *O. Lamarckiana*, since after crosses with other species or mutants it is not split off by the *laeta* hybrids, but only by those of the type *velutina*.

LUNTEREN, HOLLAND

NOTES ON AMERICAN WILLOWS. III

A CONSPECTUS OF AMERICAN SPECIES AND VARIETIES OF SECTIONS RETICULATAE, HERBACEAE, OVALIFOLIAE, AND GLAUCAE

CAMILLO SCHNEIDER

In this third article, as I said in my first paper,[1] a key will be given containing the species treated in the first two papers, and also those of the sections RETICULATAE and HERBACEAE (RETUSAE), together with a few other species the systematic position of which is not yet fully understood, but which are best placed near one of these groups. I have tried to prepare two separate keys for the determination of the male and female plants as I did in "Conspectus analyticus Salicum Asiae orientalis Himalayaeque" in SARGENT, Pl. Wils. 3:73. 1916. Typical complete specimens are not always at hand, and without them even such a good key as that given by COVILLE (in Proc. Wash. Acad. Sci. 3:300. 1901) to the Alaskan willows is insufficient to determine species. To make a key to the sections only proves likewise of little value owing to the great difficulty of exact limitation of the groups, as I shall explain later.

Clavis specierum

I. SECUNDUM SPECIMINA FEMINEA

A. Ovaria (pedicellis inclusis) etiam juvenilia glaberrima.

I. Folia utrinque concoloria, viridia et stomatifera, minima vel parva; amenta serotina, vulgo pauciflora; bracteae pl.m. concolores, flavescentes vel violascentes, vix vel sparse brevi-pilosae; fruticuli minimi prostrati (sed vide 7. *S. Peasei*).

Folia semper crenato-dentata, utrinque tenuiter reticulata, anni praeteriti nunquam persistentia.

Ramuli floriferi tenuissimi, breves, fere semper bifoliati; bracteae flavescentes, sparse pilosae.....8. *S. herbacea*

Ramuli floriferi crassiores, longiores, 2–4-foliati; bractae fuscescentes, albido-pilosae.....7. *S. Peasei*

[1] BOT. GAZ. 66:118. 1918.

Folia integerrima, adulta sicca per secundum annum vel diutius persistentia.

Nervi laterales foliorum utrinque pl.m. elevati, venulae etiam prominulae (confer etiam 10. *S. phlebophyllam*, cujus fructus interdum glaberrimi sunt)..9. *S. rotundifolia*

Nervi laterales foliorum superne tenuissime incisi, subtus prominentes, venulae haud visibiles..11. *S. Dodgeana*

II. Folia discoloria, subtus distincte pallidiora, pl.m. glaucescentia (saepissime pruinosa); fruticuli repentes vel parvi, erecti.

Bracteae concolores, flavescentes, glabrae vel sparse tantum ciliatae.

Fruticuli parvi, erecti, ramis non prostratis et radicantibus; styli distincti, quam stigmata brevia bifida longiores, haud vel tantum apice fissi; folia circiter 3–5plo longiora quam lata.

Folia superne cito margine excepto glabra, stomatifera 21. *S. chlorolepis*

Folia superne infimis exceptis pl.m. villosula, estomatifera.....22b. *S. brachycarpa* var. *glabellicarpa*

Fruticulus depressus, ramis prostratis radicantibus; stylus brevissimus, vulgo bifidus stigmatibus bifidis haud longior; folia satis crassa, vix 1½plo longiora quam lata 3. *S. leiolepis*

Bracteae pl.m. bicolores, ad apicem vel fere totae fuscae, pl.m. longe sericeo-pilosae; fruticuli parvi, prostrati, ramis radicantibus.

Ovaria etiam juvenilia distincte pedicellata; pedicellus fructuum glandulam late ellipsoideo-rectangularem vel subquadratam circ. 2plo superans; stylus distinctus, apice bifidus stigmatibus brevibus oblongis bifidis subduplo longior; folia superne haud stomatifera, margine vulgo sparse et saepe indistincte denticulata 18. *S. arctophila* f. *lejocarpa*

Ovaria subsessilia vel breviter pedicellata, pedicello etiam fructuum quam glandula pl.m. breviore vel vix sublongiore.

Folia pl.m. glanduloso-crenato-denticulata (saltem ad medium et apicem), rarius subintegerrima, superne sto-

matifera, adulta marcescentia partim diu persistentia; stipulae saepe distinctae; stylus apice bifidus stigmatibus brevissimis bifidis 2–2½plo longior....6. *S. Uva-ursi*
Folia integerrima, rarissime basim versus paucidentata; stipulae nunquam distinctae.

Amenta cylindrica, 3–4plo longiora quam lata, 3–5 cm. longa (saltem basi) sublaxiflora; folia majora ultra 3 cm. longa, superne non stomatifera
15. *S. arctica* f. *glabrata*
Amenta etiam fructifera vix ad ½plo longiora quam lata, densiflora; folia etiam maxima vix ultra 2.5 cm. longa vel superne stomatifera.

Stylus satis brevis, stigmatibus mediocribus vix longior; fructus maturi vulgo pl.m. glaucescentes; folia superne (in var. *camdensi* excepta) haud stomatifera, matura subcrassa, subtus conspicue elevato-reticulata.....16. *S. ovalifolia*
Stylus elongatus tenuis, stigmatibus angustis longis saepe pl.m. longior; fructus maturi vix glaucescentes; folia superne stomatifera, matura tenuiora, subtus vix conspicue reticulata....17. *S. stolonifera*

B. Ovaria (interdum tantum partim vel nonnisi pedicelli) pl.m. dense pilosa; fructus saepe glabriores vel partim glabri.

I. Folia (sub anthesi perfecte evoluta) utrinque concoloria, viridia, aequaliter stomatifera, integerrima; fruticuli minimi repentes.

Ramuli floriferi breves, tenuissimi, vulgo 2-foliati; folia utrinque tenuiter reticulata, adulta sicca non persistentia; amenta pauciflora, vel multiflora; ovaria interdum tantum ad apicem pilosa, pl.m. sessilia; stylus distinctus, stigmatibus vulgo longior; glandula 1.....5. *S. polaris*
Ramuli floriferi 2–4-foliati; folia adulta marcescentia parenchymate evanescente plures annos persistentia; amenta pluriflora, cylindrica; ovaria saepius distinctius pedicellata; glandula etiam dorsalis interdum adest (confer etiam 9. *S. rotundifoliam* f. *pilosiusculam*).....10. *S. phlebophylla*

II. Folia subtus discoloria, pallidiora vel pl.m. glaucescentia, vulgo pruinosa, vel plantae aliis signis diversae.

a. Folia circumcirca satis dense minute glanduloso-serrata, obovata, fere glabra; stipulae distinctae, lanceolatae, serratae; amenta pedunculo excluso 5–6 cm. longa, 10–15 mm. crassa; stylus distinctus, stigmatibus circ. 2plo longior; planta prostrata.....29. *S. Chamissonis*

b. Folia integerrima vel pl.m. crenato-denticulata, vel plantae aliis signis diversae.

1. Amenta serotina, pseudoterminalia, anguste cylindrica vel minima pauciflora, pedunculis nudis iis saepe aequilongis suffulta; bracteae breviter (rarius longius) pilosae, pl.m. concolores, flavescentes vel violascentes; ovaria sessilia vel subsessilia; styli (*S. venusta* excepta) brevissimi vel nulli, stigmata brevia vel brevissima; glandulae 2 vel interdum plures pseudodiscum lobulatum formantes; folia satis crassa, superne pl.m. inciso-reticulata, rugosa, haud stomatifera (*S. venustae?*), subtus distincte elevato-reticulata, vulgo pl.m. longe petiolata.

Folia coriacea, pleraque vix longiora quam lata vel ultra 4 cm. longa, superne conspicue inciso-reticulata, rugulosa; bracteae intus pl.m. brevipilosae vel utrinque sericeae.

Frutex prostratus; folia cito glaberrima vel rarius pilis paucis sericeis obsita, vix ad 5.5:5 cm. magna; petioli elongati, ad 3 cm. longi; bracteae intus tantum brevipilosae; fructus vix ultra 4.5 mm. longi

1. *S. reticulata*

Frutex prostratus vel erectus; folia etiam adulta subtus dense sericea vel majora, oblongiora et margine pl.m. distincte crenulata, vel petioli breves gemmis vix longiores: bracteae utrinque pl.m. sericeo-pilosae; fructus 5–7 mm. longi.....2. *S. vestita*

Folia tenuiora (chartacea), minima vel distincte longiora quam lata, vix ultra 3.5:2 cm. magna, superne vix vel indistincte inciso-reticulata; bracteae glabrae vel tantum margine basique pilosae, rarius etiam extus pl.m.

pilosulae; frutices prostrati, interdum minimi suffruticulosi (confer etiam 31. *S. venustam* cujus specimina nondum vidi et quae stylo elongato filiformi fusco distincta dicitur; tantum a Sitka reportata)

4. *S. nivalis* et var. *saximontana*

2. Amenta coetanea, rarius pl.m. serotina, lateralia, ovata vel cylindrica, multiflora, rarius parva et pauciflora, sed pedunculi semper foliati; folia pl.m. tenuiter papyracea, superne nunquam inciso-reticulata.

a) Bracteae bicolores, apice vel pro parte maxima fuscae, versus apicem longe sericeae (id est pilis longis sericeis quam bractea vix vel paullo brevioribus instructae), interdum apice tantum ciliatae; fruticuli fruticesque parvi ramis ut videtur semper prostratis (confer etiam 28. *S. lingulatam* speciem valde incertam).

Stylus sub nullus, quam stigmata divaricata bifida duplo brevior; amenta parva circ. 10 mm. longa, ovoidea vel ovato-globosa; bracteae atrae, extus sparse sericeae vel partim glabrae; ovaria breviter pedicellata, albo-sericeo-villoscula; folia ovata vel late lanceolata, obtusa, rigida vix ad 12 mm. longa....30. *S. glacialis*

Stylus semper distinctus, stigmatibus aequilongus vel vulgo longior.

Folia sicca anni praeteriti pl.m. persistentia, lingulata, lineari- vel anguste oblanceolata (vel lanceolata apice subplicato-acuminata), vix ultra 18:5 mm. magna, superne stomatifera, subtus paullo pallidiora; amenta parva, etiam fructifera vix ad 2.5:0.9 cm. magna pedunculis 0.5–3 cm. longis exclusis; bracteae laxe sericeae, extus saepe glabrescentes; (ovaria et) fructus subsessiles, circ. 4–5.5 mm. longi, pl.m. villoso-tomentosi.....12. *S. cascadensis*

Folia adulta haud persistentia et plantae aliis signis diversae.

Stigmata linearia, elongata, stylo tenui satis longo pl.m. 2–3plo breviora; ovaria fructusque saepe

tantum ad apicem sparse pilosi, ceterum ut supra sub *S. stolonifera* indicata

17. *S. stolonifera* f. *subpilosa*

Stigmata brevia vel oblonga sed vix linearia vel plantae aliis signis diversae.

Folia subtus (in sicco) paullo pallidiora (haud distincte glaucescentia vel albescentia), leviter elevato-nervata sed vix reticulata, laevia, petiolis vix 5–6 mm. longis instructa, vulgo lanceolata vel elliptico-lanceolata, utrinque acuta vel apice obtusa, superne stomatifera et interdum fere inciso-nervata, etiam maxima vix ad 4:1.8 cm. magna, integerrima (rarissime versus basim parce denticulata); stipulae nullae vel minimae, caducae; amenta (pedunculo excluso) 2–4.5 (in var. *caespitosa* interdum ad 6) cm. longa et fructifera ad 1.3 cm. crassa; ovaria subsessilia, dense sericeo-villosa; stylus distinctus, saepe apice breviter bifidus, stigmatibus oblongis bifidis vulgo duplo rarius 3plo longior; glandula ventralis oblonga, ovoideo-conica; fructus pedicello brevi glandulam siccam subaequante excluso 4–5 mm. longi; fruticulus ramulis hornotinis flavescentibus tenuibus satis brevibus. . .13. *S. petrophila*

Folia subtus distincte discoloria, glaucescentia, pruinosa, superne haud stomatifera vel majora, diversiformia vel plantae aliis signis diversae.[2]

Glandula ventralis satis brevis et lata, vix duplo altior quam lata, apice late truncata, pedicello fructuum duplo brevior; amenta submatura vel fructifera (3–)5–10:1.2–1.6 cm. magna; ovaria tenuiter villoso-tomentella; styli distincti, apice bifidi, stig-

[2] It is difficult to indicate in such a key the differences between *S. petrophila* and *S. anglorum* and its different forms with sufficient clearness. See my remarks under those species in my first paper (*l.c.*).

matibus oblongis bifidis paullo vel duplo (rarius fere triplo) longiores; fructus pedicello excluso 6.5–8 mm. longi; folia superne estomatifera, margine vulgo partim sparse et saepe obsolete denticulata, forma variabilia, majora latiora ad 3–4(–5):2.5(–3) cm. magna; frutex procumbens ramulis saepe satis elongatis, 2–3 mm. crassis (si glandula est brevis et lata sed pedicello brevior, confer 18a. *S. hudsonensem*) . . . . . 18. *S. arctophila*

Glandula oblonga, vulgo $2\frac{1}{2}$–4plo longior quam lata et pedicelli etiam fructuum quam glandula pl.m. breviores vel rarius sublongiores vel plantae aliis signis diversae.

Amenta fructifera ellipsoideo-globosa, circ. 1–1.5:1.5 cm. magna; folia subcoriacea, late ovalia vel obovato-rotunda, vix ultra 1.8:1.5 cm. magna, superne estomatifera, in sicco tenuiter reticulata, subtus valde elevato-reticulata, utrinque (saltem initio) ut ramuli novelli villosula

16b. *S. ovalifolia* var. *pubescens*

Amenta fructifera cylindrica, vulgo longiora, tenuiora; folia tenuiora vel majora, subtus nunquam conspicue elevato-reticulata.

Folia superne haud stomatifera,[3] vulgo obovata vel obovato-oblonga, apice rotundata ad subacuta vel plicato-acuta, basi sensim vel subito attenuata, obtusa vel interdum rotundata, majora satis evoluta 3–6:2–4 cm. magna; petioli 9–20 mm. longi; stipulae in ramulis vegetis ovato-lanceolatae vel lanceolatae,

[3] With the exception of *S. arctica* var. *subcordata*, which, however, is easily distinguished from any of the forms of *S. anglorum* by its much larger leaves and longer stouter catkins.

integrae vel subdenticulatae, 2–12 mm. longae; amenta sub anthesi 2.5–4 cm. longa, bracteis atris dense longe sericeis conspicua, fructifera 6:1.3 ad 9:1.8 cm. magna; ovaria sessilia vel subsessilia, sericeo-villosa; styli distincti, vulgo integri, quam stigmata oblonga bifida 2–2½plo longiores; fructus pedicello brevi glandula ⅔ ad vix breviore (rarissimo sublongiore) excepto (6–)8–10 mm. longi (confer etiam 16c. *S. ovalifoliam* var. *subarcticam* et 18a. *S. hudsonensem*)

15. *S. arctica* et varietates

Folia superne stomatifera, vulgo minora, valde variabilia (vide formas sub *S. anglorum* enumeratas); petioli vix ultra 10 mm. longi; stipulae nullae vel minores; amenta etiam fructifera vix ad 5.5:1.5–1.8 cm. magna, saepe distincte minora, tenuiora; fructus ad 7–8 mm. longi pedicello subnullo vel glandula 2 ad ⅓plo breviore excluso (si folia sunt estomatifera sed plantae aliis signis haud diversae, confer etiam 18a. *S. hudsonensem*)

14. *S. anglorum* et varietates

β) Bracteae concolores, flavescentes, stramineae vel brunnescentes (rarius subbicolores, apice violaceae vel leviter fuscae), semper breviter sericeo-villosulae (id est pilis quam bractea brevioribus instructae), intus interdum glabratae.

(1) Petioli brevissimi, 1–2 vel vix ultra 2.5 mm. longi, gemmas bene evolutas non superantes et stipulae petiolis aequilongae vel duplo longiores; amenta florifera minima vel parva, etiam fructifera vix ad 2.5:1–1.2 (vel in *fullertonensi* ad 4:1.3) cm. magna.

Frutex prostratus ramis repentibus; folia breviter

lanceolata, elliptico-oblonga vel oblonga, utrinque pleraque acuta, 1:0.5 ad 3:0.9–1.2 cm. magna, superne vulgo sparse stomatifera; stipulae distinctae; ovaria sessilia vel subsessilia, villosulo-tomentosa; stylus satis brevis, integer vel apice bifidus stigmatibus oblongis bifidis subaequilongus; bracteae oblongae; glandula dorsalis anguste conica, interdum pl.m. bifida, iis duplo brevior; fructus subsessiles, 4.5–6 mm. longi

19. *S. fullertonensis*

Frutices parvi erecti, ramis saepe satis brevibus subtortuosis divaricatis vel subelongatis strictioribus et plantae aliis signis diversae.

Folia superne stomatifera, oblonga, ad 3:1 cm. magna adulta etiam subtus satis glabrata; bracteae intus glabrae, late ovales vel obovales; stylus distinctus quam stigmata vulgo ultra duplo longior; ovaria sessilia, saepe infra medium glabra; ramuli hornotini satis glabrescentes.....21b. *S. chlorolepis* var. *antimima*

Folia superne haud stomatifera, magis (saltem subtus) villosa vel sericeo-villosa; ramuli hornotini semper pl.m. dense villoso-tomentosi; bracteae vulgo utrinque pilosae vel interdum extus glabriores.

Ramuli hornotini densissime albo-sericeo-villosi; styli ovariorum sessilium, brevissimi, vix ad 0.5 mm. longi, stigmatibus brevibus; folia oblongo-lanceolata vel anguste ovato-lanceolata, apice (infimis exceptis) pl.m. acuta, basi rotundata vel obtusa, 1.5:0.5 ad 3.5:0.9–1 cm. magna, novella utrinque sericeo-villosa; species satis imperfecte cognita.....20. *S. niphoclada*

Ramuli hornotini vel vulgo tantum novelli minus dense griseo-vel subflavescenti-sericeo-villosi; styli distinctiores, stigmatibus aequi-

longi vel saepe distincte longiores; folia elliptico-oblonga, oblanceolata, rarius ovato- vel elliptico-lanceolata, interdum obovato-oblonga, apice obtusiuscula vel subito breviter acuta, basi late cuneata vel obtusa, rarius rotundata ad subcordata, sub anthesi saepe minima vel parva, subspathulata vel lineari-lanceolata, ad 2.5–3:(0.6–)1 vel 3.4:0.8 vel ad 3:1.1 (maxima ad 4.5:1.2) cm. magna; amenta sub anthesi vix ultra 10:4 mm., fructifera 1.5:0.8 ad 2.5:1(1.2) cm. magna; fructus subsessiles vel pedicello glandula vulgo duplo breviore suffulti, 5–7 mm. longi
22. *S. brachycarpa*

(2) Petioli gemmis vel stipulis longiores, amenta etiam florifera longiora vel plantae alio modo diversae. Folia parva vel mediocra, majora apice ramulorum vulgo haud ultra 4:1.8 cm. magna, lanceolata, oblanceolata, elliptico-lanceolata, anguste elliptica ad obovato-lanceolata, rarius elliptico-vel obovato-oblonga, apice acuta vel subacuta, basi cuneata ad rotundata, integerrima (infima minima tantum brevissime glanduloso-denticulata), superne stomatifera, novella pl.m. tenuiter griseo-villosula, demum glabrescentia vel subglabrata, subtus discoloria, glaucescentia, ut superne vel densius villosula (sed pubescentia satis variabili); stipulae nullae vel valde reductae; amenta sub anthesi 8–15:5 ad 25:6 mm., fructifera ad 2–3:1–1.5 cm. magna; fructus 6.5–8 mm. longi, pedicello brevi quam glandula duplo breviore (rarius ea subaequilongo) excluso; ramuli novelli griseo-villosuli vel subtomentosi, annotini saepe subglabri, purpurasentes vel fere nigro-castanei vel ut vetustiores epidermide griseo-flavescente pl.m. secedente obtecti (conf. etiam 23. *S. desertorum*, speciem tantum incomplete cognitam).....24. *S. pseudolapponum*

Folia majora vel latiora vel superne haud stoma-

tifera vel amenta fructifera majora et fructus 8–10 mm. longi pedicellis glandulam ad duplo superantibus.

Folia superne haud stomatifera.[4]

Fructus pedicello subnullo vel brevi glandulam haud superante suffulti, 6–8(–10) mm. longi; amenta sub anthesi 1–2.5:0.7 cm., fructifera ad 3–5:1.2–1.5 cm. magna pl.m. densa; folia (var. *atra* excepta) tantum $\frac{1}{2}$ ad $2\frac{1}{2}$plo longiora quam lata, elliptica, ovalia, obovato-elliptica, obovato-oblonga, vel ovato-elliptica, apice pl.m. obtusa vel breviter acuta, basi obtusa vel late cuneata ad subcordata, 3:2 vel 3.5:2.5 vel 4.5:2–2.3 ad 6:2.8 cm. magna, novella pubescentia (minimis infimis exceptis) pl.m. villosa induta, superne glabrescentia, rarius adulta utrinque glaberrima.....26. *S. cordifolia*

Fructus pedicello glandulam vulgo $\frac{1}{4}$ ad 2plo superante instructi, 7–8(–9) mm. longi; amenta sub anthesi 2–4:0.8–1 cm., fructifera 3.5–7:1.5 cm. magna, basi saepe satis laxiflora; folia vulgo $2\frac{1}{2}$ ad ultra 4plo longiora quam lata, lanceolata, oblanceolata, elliptico-oblonga, obovato-oblonga vel obovato-elliptica, apice obtusa vel vulgo acuta vel fere breviter acuminata, basi obtusa vel subito sensimve cuneata, mediocra 4.5:2 ad 5:1.5 vel 7:2.3 cm. magna, novella pubescentia satis sericea induta, superne paullo vel omnino glabrescentia, subtus saepe glabriora 25. *S. glaucae* varietates

Folia superne stomatifera, ceterum ut in vol. 66, p. 349 descripta.....27. *S. anamesa*

[4] Owing to the variability of the nos. 24–26 and our insufficient knowledge of the Greenland forms I cannot avoid using this anatomical character in distinguishing *S. anamesa* from the other two species. But *S. anamesa* is apparently a species met with only in Greenland, and therefore the arrangement of the key will not be inconvenient to most of the students of American willows.

2. SECUNDUM SPECIMINA MASCULA[5]

A. Filamenta omnino glabra

I. Stamen unicum (rarissime stamina 2 adsunt); bracteae pl.m. purpurascentes et apice atrae, pilis longis argenteis sericeae; folia adulta glabra, discoloria, superne pl.m. nitida et stomatifera, pl.m. crenato-denticulata, rariter ad 2.5 cm. longa; frutex depressus vel prostratus . . . . . 6. *S. Uva-ursi*

II. Stamina semper 2.

a. Fruticuli minimi, suffruticosi, ramulis tenuissimis, fere semper radicantibus; folia utrinque concoloria v. subconcoloria, etiam superne stomatifera, minima vel parva, rarius ultra 25 mm. longa latave; amenta serotina, tenuia, pauci- (rarius multi-) flora.

Folia utrinque obtusa vel acutiuscula (cuneata), integra, nervis primariis superne tenuissime incisis subtus prominentibus, ceterum enervia, 5–8:3–4 mm. magna; amenta 3–5-flora, rhachi bracteisque concoloribus glabris vel parcissime pilosis; glandulae 2 . . . . . 11. *S. Dodgeana*

Folia utrinque rotundata vel basi cordata vel utraque facie distincte (sed saepe tenuiter) reticulata.

Ramuli breves floriferi fere semper bifoliati; folia adulta sicca haud per secundum annum persistentia; ramuli vetustiores 1–2 mm. crassi.

Bracteae glabrae subglabraeve, concolores, flavescentes vel violascentes; glandulae 2; folia semper crenato-serrata . . . . . 8. *S. herbacea*

Bracteae sericeo-villosulae, fuscae, pl.m. bicolores; glandula 1, ventralis; folia saepissime integerrima 5. *S. polaris*

Ramuli floriferi 2–5-foliati; folia adulta sicca pl.m. marcescentia, fuscescentia et per secundum annum persistentia, integerrima; glandulae fere semper 2 (in *S. cascadensi* ut videtur tantum 1).

[5] Of the following species the male plant is still unknown: *fullertonensis*, *hudsoniensis*, *leiolepis*, *lingulata*, *Peasei*, and *venusta*.

Amenta minima, 3–8-flora; folia vulgo orbicularia vel late ovalia, utrinque rotundata, vix ad 11:10 mm. magna, adulta sicca anno secundo decidua
9. *S. rotundifolia*

Amenta multiflora, ad 23 mm. longa; folia adulta plures annos persistentia vel pl.m. lineari-lanceolata.
Folia elliptica, obovato-oblonga vel late spathulata, apice rotundata vel breviter acuta, basi cuneata, subtus vix pallidiora, ad 15:9 mm. magna ..... 19. *S. phlebophylla*
Folia lingulata, lineari vel anguste lanceolata (vel oblanceolata), apice vulgo subplicato-acuminata, ad 18:5 mm. magna, subtus subpallidiora
12. *S. cascadensis*

b. Fruticuli vel frutices vel folia discoloria vel plantae alio modo diversae.

1. Bracteae pl.m. discolores, apice vel pro parte maxima fuscae, versus apicem longe sericeae (pilis longis sericeis quam bractea vix vel paullo brevioribus instructae), interdum apice tantum longe ciliatae; fruticuli vel frutices parvi, ramis prostratis, pl.m. radicantibus vel subterraneis, ramulis tantum floriferis pl.m. adscendentibus, vix ad 15–20 cm. altis (vel in *S. arcticae* formis interdum altioribus); si folia sunt tenuiter sed dense et acute glanduloso-serrulata, vide
29. *S. Chamissonis*

α) Folia subtus (in sicco) paullo pallidiora, leviter elevato-nervata, sed vix reticulata ceterum ut in p. 56 descripta; amenta 1–2.5 cm. longa, vix ad 1 cm. crassa, pluri- vel multiflora; glandula ventralis pl.m. elongato-conica, dorsalis saepe nulla
13. *S. petrophila*

β) Folia subtus distincte discoloria, glaucescentia vel albescentia, adulta pl.m. reticulata vel longius petiolata vel majora et forma diversa (specimina mascula specierum sequentium sine speciminibus

femineis accurate discernere saepe impossibile est).[6]

(1) Glandula ventralis satis brevis lataque, vix 2plo longior quam lata, apice late truncata, quam bractea obovata vulgo $2\frac{1}{2}$–3plo brevior;[7] amenta 2–2.5:0.8–1 cm. magna; folia tantum novella subtus sparse sericea, cito glabra, superne estomatifera, ceterum ut in p. 57 descripta; petioli vix ultra 8 mm. longi . . . . . 18. *S. arctophila*

(2) Glandula ventralis pl.m. anguste ovato-rectangularis vel anguste conica, apice saepe leviter incrassato truncata, quam bractea vix duplo brevior vel folia superne stomatifera, semper integerrima vel plantae aliis signis diversae.

Amenta perfecte evoluta vix ultra 1.5:0.8 cm. magna; folia matura satis crasse papyracea, subtus pl.m. perspicue et anguste reticulata, vulgo elliptica, late elliptica, obovalia vel rotundata, vix ultra 2.5:1.2–2 cm. magna; stipulae nullae (vel minimae punctiformes) (si folia superne stomatifera confer. etiam 14. *S. anglorum* formas et 30. *S. glacialem* speciem valde incomplete cognitam).

Folia superne pl.m. stomatifera; ramuli annotini hornotinique vulgo breves; rami saepe stolones subterraneos tenues vix ad 1 mm. crassos emittentes. . 17. *S. stolonifera*

Folia superne haud (var. *camdensis* excepta) stomatifera; ramuli hornotini annotinique pl.m. elongati, rami ut videtur haud stoloniferi. . . . . 16. *S. ovalifolia*

[6] Of course if their exact locality is known, we shall certainly be able to determine even young male branchlets.

[7] The shape of the ventral gland is often rather variable in any species because the gland may be more or less lobate, bifid, or bipartite.

Amenta perfecte evoluta vulgo ultra 1.5 et ad 4–5 cm. longa et 1–1.3 cm. crassa, bracteis longe et dense pilosis satis sericea; folia matura majora vel tenuiora et subtus vix reticulata, juvenilia magis sericea vel sericeo-villosula, vel stipulae (saltem in ramulis vegetis) pl.m. distincte evolutae.

Folia superne haud stomatifera (var. *subcordata* excepta quae foliis amentisque maximis a *S. anglorum* valde differt) ceterum ut in p. 56 descripta; amenta 1.5–5:1.3 cm. magna; ramuli pedunculiferi vulgo 2–4 mm. crassi (confer etiam 16c. *S. ovalifoliam*, v. *subarcticam*)

15. *S. arctica*

Folia superne stomatifera, ceterum ut in p. 56 descripta; ramuli pedunculiferi 1 ad vix 2 mm. crassi (confer etiam 27. *S. anamesam* e Groenlandia) . . 14. *S. anglorum*

2. Bracteae pl.m. concolores, flavescentes et fere glabrae vel stramineae flavobrunnescentesve et pl.m. breviter sericeo-villosae (pilis quam bractea brevioribus instructae) vel subdiscolores sed tantum villosae vel pilis sericeis vulgo tenuissimis praeditae; frutices prostrati vel saepe erecti, 0.3–1 m. alti.

Amenta minima vel parva, 5–10 mm. longa vel in *niphoclada* ad 22:4 mm. magna et laxiflora; petioli vix ultra 2 mm. longi.

Folia parva, vix ad 2.5 cm. longa et ad 1.4 cm. lata, superne stomatifera, stipulae ut videtur nullae; bracteae flavescentes vel stramineae, subglabrae vel extus dense breviter pilosae; glandulae 2

21. *S. chlorolepis*

Folia saepe ad 4 cm. longa, superne haud stomatifera; stipulae vulgo evolutae; bracteae stramineae, utrinque brevipilosae; glandulae 2

20. *S. niphoclada*

Amenta 1.2–3.5 cm. longa, ultra 5 mm. crassa; petioli vulgo ultra 2 mm. longi; confer formas diversas sub 24. *S. glauca*, 25. *S. cordifolia*, et 26. *S. anamesa* enumeratas.

B. Filamenta pl.m. pilosa (interdum ima basi tantum pilis paucis instructa)

I. Amenta serotina, pseudoterminalia, anguste cylindrica vel minima pauciflora, pedunculis nudis iis saepe subaequilongis suffulta; bracteae breviter (rarius longius) pilosae, pl.m. concolores, flavescentes vel violaceae; folia satis crassa, superne saepe pl.m. inciso-reticulata, haud stomatifera, subtus distincte elevato-reticulata, pl.m. longe petiolata; glandulae 2 vel interdum plures pseudodiscum lobulatum formantes.

Bracteae intus pl.m. brevipilosae vel utrinque sericeae; folia coriacea, pleraque vix longiora quam lata vel ultra 4 cm. longa, superne conspicue inciso-reticulata, rugosa.

Frutex prostratus; folia cito glaberrima vel rarius pilis paucis sericeis obsita, vix ad 5:5.5 cm. magna et petiolis elongatis ad 3 cm. longis instructa; bracteae intus tantum brevi-pilosae; antherae violaceae..... 1. *S. reticulata*

Frutex prostratus vel erectus; folia etiam adulta subtus dense sericea vel majora, magis oblonga, margine distincte crenata, vel petioli breves gemmis vix longiores: bracteae utrinque pl.m. sericeae; antherae flavae..... 2. *S. vestita*

Bracteae glabrae vel tantum parce ciliatae; folia tenuiora, minima vel distincte longiora quam lata, vix ultra 3.5:2 cm. magna, superne vix vel indistincte inciso-reticulata; frutices prostrati, interdum minimi, suffruticulosi

4. *S. nivalis* et var. *saximontana*

II. Amenta coetanea, rarius serotina, lateralia, ovata vel cylindrica, semper multiflora, sed interdum parva, pedunculis semper foliatis.

Folia brevissime petiolata, petiolis vix ultra 2.5 mm. longis vel quam gemmae evolutae pl.m. brevioribus, ceterum ut in p. 58 indicata; amenta sub anthesi 5–10(–15):2–8(–9) mm. magna; antherae minimae, ellipsoideo-globosae

22. *S. brachycarpa*

Folia distinctius petiolata; amenta vulgo ultra 15 mm. longa, crassiora vel antherae magis ellipsoidales oblongiores et folia superne pl.m. stomatifera (specimina mascula specierum sequentium sub anthesi sine foliis perfecte evolutis accurate discernere saepe impossibile videtur; confer etiam 28. *S. lingulatam* speciem incertam alaskanam).

Folia superne pl.m. (interdum tantum sparse secundum nervos) stomatifera, vulgo oblonga, $2\frac{1}{3}$–4plo longiora quam lata, sed vix ultra 4:1.8 cm. magna.

Amenta pedunculis vix ad 1 cm. longis suffulta, 8–15:7 mm. magna, densiflora; folia pedunculorum (saltem subtus) dense breviter sericea vel villosula (confer etiam 27. *S. anamesam* e Groenlandia).... 23. *S. pseudolapponum*

Amenta saepe longius pedunculata, 1.5–3.5:0.8–0.9 cm. magna et basim versus laxiflora vel folia pedunculorum etiam subtus glabra vel subglabra..... 24. *S. desertorum*

Folia superne haud stomatifera, majora, latiora vel longiora.

Amenta deflorata ad fere 3.5 cm. longa, basi vulgo pl. m. laxiflora vel folia pedunculorum satis oblonga, circ. $2\frac{1}{2}$–$3\frac{1}{2}$plo longiora quam lata..... 25. *S. glaucae* varietates

Amenta deflorata vulgo haud ultra 2(–2.5) cm. longa, etiam basi densiflora vel folia pedunculorum latiora brevioraque vix ad $2\frac{1}{2}$plo longiora quam lata

26. *S. cordifolia*

Enumeratio sectionum specierumque

I have omitted from the keys and the following enumeration the well known and easily recognizable *S. candida* Flügge which BALL (1909) includes in his section ARCTICAE, because I attribute to it a different systematic position.

Sect. I. RETICULATAE[8] Fries in Sylloge Pl. Nov. Soc. Ratisb. 2:38 (Consp. Disp. Salic. Suec.). 1828, quoad *S. reticulata;* for

[8] There is the older name *Chamaetia* given by DUMORTIER in Bijdr. Natuurk. Wetensch. 1:56 (Verh. Gesl. Wilgen 15) 1835 to a group, including *S. retusa*, *S. herbacea*, and *S. reticulata*. Unfortunately neither the International Rules nor the Philadelphia Code contains a precise rule in regard to the application of names of sections or similar groups. I do not accept DUMORTIER's name because in his paper he proposes two very different arrangements, and he does not in my opinion make a definite statement.

further literature see SCHNEIDER in SARGENT, Pl. Wils. 3:146. 1916. —This section, which is represented in America by the following 4 species, is a well defined group. The rather exceptional position of *S. reticulata* among the other willows, which once led A. KERNER to propose the new genus *Chamitea* for it, becomes less marked by the addition of the American *S. nivalis;* and the characters of the RETICULATAE are further changed by the inclusion of *S. leiolepis* with glabrous ovaries. *S. glacialis* referred by RYDBERG to this section is a very imperfectly known species, of which the systematic position is still doubtful.

1. S. RETICULATA L., Sp. Pl. 2:1018. 1753.—*S. reticulata* α *glabra* Trautvetter in LEDEB., Fl. Alt. 291. 1833.—*S. reticulata* b *normalis* And. in Öfv. K. Vet.-Akad. Förh. 15:133 (Bidr. Känned. Nordam. Pilarter). 1858.[9]—*S. reticulata* α *typica* 1. *glabra* And. in DC., Prodr. 16²:301. 1868.—This willow has the most extensive range of all the known species. In Europe it is reported from the high Pyrenees through the whole range of the Alps to the mountains of Croatia, and northward to Scotland, Scandinavia, Iceland, Spitzbergen, and Arctic Russia, while in Asia it is found on the high mountains from the Ural to Kamchatka and in the Arctic zone from Taimyr Peninsula to the Bering Strait. According to LANGE it does not occur in Greenland. In North America I have seen specimens from southern Labrador, western Newfoundland, the northern shores of the Hudson Strait, and the western shore of Hudson Bay to the Coronation Gulf and Bernard Harbor (114°46′ W. long.), and west of 135° W. long. from the Yukon Territory (King Point and Herschel Island to Lake Bennett) and from Alaska. Here it stretches, as COVILLE has said, over the Arctic zone, but including the extreme north (Camden Bay), and southward it occurs at timber line on the mountains from the Juneau region to Kodiak Island, and westward to the Aleutian, Pribilof, and St. Matthew Islands. There is also a specimen from the "Rocky Mountains" (no. 85 Herb. H.B.T., ex Herb. Torrey in N.; m., f.), the exact locality of which is unknown to me. No. 86 also

[9] The same article has been published with slight alterations in the same year in Proc. Amer. Acad. 4:50 (Salic. Bor.-Am.) and in Walper, Ann. Bot. 5:744. I do not always repeat these quotations.

of the same collection is a female specimen which seems to represent a very small form of *S. reticulata* somewhat similar to *S. nivalis*, but showing a distinct reticulation of the leaves. It needs further observation and has already been mentioned by RYDBERG (1899), who also cites a specimen of MACOUN (18849, O.) from Silver City in the Rockies, a locality I have not yet been able to identify. Otherwise it is replaced in the Rockies by *S. nivalis* and var. *saximontana*.

The name *S. orbicularis* has been given by ANDERSSON (in DC., Prodr. 16²:300. 1868) to the *S. reticulata* "in Kamtschatka et in America boreali-occidentali ut ad Sitchka et Unalaschka." RYDBERG (in Bull. N.Y. Bot. Gard. 1:260. 1899) accepted this name for almost all the American material of *S. reticulata*, citing only three specimens "which may be referred" to LINNAEUS' species. But I agree with COVILLE (in Proc. Wash. Acad. Sci. 3:342. 1901) that the distinguishing characters assigned by ANDERSSON as well as those given by RYDBERG are insufficient "to see in our American plant a species distinct from the European." If we wish to distinguish the form with more or less orbicular leaves we may use the name *S. reticulata subrotunda* Seringe (Essai Mon. Saules Suisse 29, 1815) based on *S. reticulata* Hoffmann (Hist. Salic. 1: *pl. 25, fig. 3.* 1787). Other variations of this species which have been observed in the Old World are not represented among the American material before me and are not mentioned by previous authors.

2. S. VESTITA Pursh, Fl. Amer. Sept. 2:610. 1814.—*S. reticulata* α *vestita* And. in Öfv. K. Vet.-Acad. Förh. 15:133. 1858, excl. specim. e Siberia et Helvetia.—*S. vestita* α *humilior* And. in DC., Prodr. 16²:300. 1868, excl. specim. altaica.—The range of this well known species extends (including the form mentioned later) in the east from northern Labrador southward to western Newfoundland, Anticosti, and the Gaspé Peninsula, and I saw it also from the west shore of Hudson Bay (Churchill, lg. *J. M. Macoun*, no. 79143, O., m., f.; Cor., G.), while in the west it reaches its northern limit in the Rockies of Alberta and British Columbia toward the 52d parallel, extending southward to northwestern Montana and the Wallowa Mountains in eastern Oregon. The western forms have been called *S. Fernaldii* by BLANKINSHIP (in Mont. Agric. Coll. Sci. Stud.

1:46. 1905), which name is adopted by RYDBERG (Fl. Rocky Mts. 198. 1917), although he says "perhaps not distinct from the eastern *S. vestita* Pursh." It certainly cannot be distinguished specifically, and if we intend to apply a special denomination to the more erect form with rather "thinner, narrower, rounded or pointed leaves," we have to use the name var. *erecta* And. (in DC., Prodr. 16²:300. 1868). The aments are usually longer than in the eastern form, but there are specimens before me from Alberta (lg. REHDER and also JACK) with the same short fruiting catkins. Professor FERNALD kindly pointed out that the shape of the capsules of typical *vestita* is more ovoid-conical, with a rather pointed apex, while it is more ovoid-ellipsoid, with an obtuser apex, in var. *erecta*. There seem to be rather intermediate forms, but as a whole this character may be taken for the best one to distinguish these eastern and western forms.

Another form has been collected by FERNALD and ST. JOHN in western Newfoundland which seems closely connected with the next species, but its description has not yet been published and it needs further observation.

3. S. LEIOLEPIS Fernald in Rhodora 16:178. 1914.—This is a very peculiar species, which was discovered by FERNALD and ST. JOHN on the Table Mountain, Port à Port Bay, in western Newfoundland, July 17, 1914, on "mossy knolls on the limestone tableland, alt. 200–300 m." (no. 10825, fr.; G.). In habit and foliage it closely simulates, as the author said, *S. reticulata* and the most dwarfed alpine extreme of *S. vestita;* but it differs "from both in the glabrous scales and capsules; also from *S. reticulata* in its short peduncles and thick fruiting aments, and from *S. vestita*, which is the most abundant willow of Table Mountain, in its glabrous or quickly glabrate foliage and the smaller and more slender, glabrous, greenish terminal buds." As the type specimen shows, the ovaries are sometimes sparsely pubescent, the bracts frequently provided with a few cilia, the styles very short but more or less distinct, and even the old leaves bear some hairs on the lower surface which are often rather difficult to recognize. Unfortunately the male sex is still unknown; consequently I cannot decide whether *S. leiolepis* is to be regarded as a good species or as a glabrate variety of *S. vestita*,

representing a rather dwarfed alpine form. The glabrousness or pubescence of the ovaries, a character on which usually so much reliance is placed, cannot always be taken for a decisive taxonomic character. In my notes on the species of the section OVALIFOLIAE (*l.c.*) I was able to show that many species with hairy ovaries develop a more or less glabrescent or glabrous variety, or vice versa.

4. S. NIVALIS Hooker, Fl. Bor.-Am. **2**:152. 1839; Nuttall, N. Am. Sylva **1**:77. *pl. 19, fig.* sinistra inferior. 1843; Rydberg in Bull. N.Y. Bot. Gard. **1**:262. 1899; Ball in Coult. and Nels., New Man. Rocky Mt. Bot. 139. 1909.—*S. reticulata* c *nana* And. in Öfv. K. Vet.-Akad. Förh. **15**:133. 1858, excl. specim. e Groenl. et Spitzb.—*S. reticulata β nivalis* And. in DC., Prodr. **16**²:301. 1868.—The type of this "elegant and very diminutive shrub" (NUTTALL) was collected "near the summits of the peaks in the Rocky Mountains" by DRUMMOND between lat. 52–56°. It occurs most frequently in the alpine region of the Rockies of Alberta and British Columbia, and to a certain degree also on the Tobacco Root Range (Pony Mountains), on Observation Mountain and Mt. Chauvet in southern Montana, and on the Electric Peak in northern Yellowstone Park. It is also mentioned by PIPER from Mt. Rainier, Washington. There are a few specimens from Colorado (*E. L. Greene*, no. 517, m.; G.; probably from near Golden City) and from southeastern Utah (*Rydberg* and *Garrett*, no. 8787, m., f.; N.; La Sal Mts., West Mt. Peale) which I can hardly distinguish from typical *S. nivalis*, and which, in my opinion, form connecting links between it and *S. saximontana* Rydbg. I take this last species, therefore, only for a variety of *S. nivalis*, from which it chiefly differs by the characters given later. RYDBERG himself said (1899) that *S. nivalis* "perhaps represents only the most depauperate form" of his *S. saximontana*, and he repeats in his Cat. Fl. Mont. 112. 1900 that the latter "seems to grade into *S. nivalis*," while such an accurate observer as PIPER (in Contr. U.S. Nat. Herb. **9**:216. 1906) states that "*S. saximontana* probably is not specifically distinct from *S. nivalis*."

4b. S. NIVALIS var. **saximontana,** nov. var.—*S. reticulata* Bebb in Coulter, Man. Rocky Mt. Bot. 339. 1885, non L.; Ball in Trans. St. Louis Acad. Sci. **9**:90. 1899.—*S. saximontana* Rydberg in Bull.

N.Y. Bot. Gard. 1:261. 1899; Ball in Coult. and Nels., New Man. Rocky Mt. Bot. 139. 1909.—*S. aemulans* v. Seemen in Bot. Jahrb. 29: Beibl. 65:28. 1900.—A typo praecipue differt: ramis crassioribus (interdum ad 19 mm. crassis), foliis majoribus vulgo 1.5–3.5 cm. longis et 0.8–2 cm. latis interdum minus reticulatis forma ut in typo valde variabilibus (lanceolatis saepe apice subacutis), petiolis ad 1.8 cm. longis; amentis pluri- vel multifloris masculis 7–12 mm. longis pedunculis vulgo longioribus sparse pilosis exclusis, femineis 8–18 mm. longis fructiferis fere ad 1 cm. crassis.

The type came from Gray's Peak in Colorado (lg. RYDBERG, August 1895, m.; N.), and it is most abundant in the Rockies of this state, reaching its southernmost point on the Truchas Peak of the Taos Mountains in northern New Mexico. Northward its range extends through western Wyoming, Yellowstone Park, and southern Montana to the vicinity of Laggan in Alberta and Skagit Valley in British Columbia, while toward the west it is found on Mt. Rainier in Washington, on the Strawberry and the higher Wallowa Mountains in eastern Oregon, furthermore on the East Humboldt Mountains in northeastern Nevada, and also in Boxelder and Utah counties and on the La Sal Mountains in Utah.

Sect. 2. HERBACEAE Borrer in Hooker, Brit. Fl. 432. 1830 (Sect. CHAMAETIA Dumortier, pro parte, see note 8 on p. 43; sect. RETUSAE Kerner in Verh. Zool. Bot. Ges. 10:195 [Niederöstr. Weid.]. 1860; for further literature see SCHNEIDER in Sargent, Pl. Wils. 3:142. 1916).—As I have already explained (*l.c.* 143), it seems to me impossible to separate the sect. RETUSAE from the HERBACEAE, but there are the following species which have been added to this group or might be regarded as closely related to its members: *S. cascadensis*, *S. Dodgeana*, *S. glacialis*, *S. Peasei*, *S. polaris*, *S. phlebophylla*, *S. rotundifolia*, and *S. Uva-ursi*. Of these species *S. rotundifolia* seems to show the most intimate affinity with *S. herbacea*, but it is distinguished by the persistent leaves, a character also found in *S. phlebophylla*, *S. cascadensis*, and *S. Uva-ursi*. From the last three species *S. Uva-ursi* seems to be widely separated by its bicolor leaves and the single stamen of the male flowers, while on the other hand they all have bicolor or fuscous bracts which are concolor, greenish or yellowish (or partly purplish

toward the apex) in *S. herbacea*, *S. Dodgeana*, and *S. retusa*. The systematic position of *S. polaris* is even more puzzling. Some authors are inclined to regard it as nothing but a variety of *S. herbacea* because the vegetative characters of both are so similar; but if we base our opinion on the flowers we may come to a very different conclusion; and in Sargent, *l.c.* 319, I have included this species in the sect. MYRSINITES Borrer. If we pay much attention to the presence or absence of a dorsal gland in the male flowers, we might also refer *S. Uva-ursi* to this section, but this willow occupies a rather unique position among the American species. With my present knowledge I deem it best to leave the question of the correct limitation of this section and of the true systematic position of these species undecided until I have had opportunity to discuss this problem with such an eminent salicologist as S. J. ENANDER, who is preparing a monograph of the whole genus. I have already published (Oestr. Bot. Zeitschr. 65:273. 1915) a short note on the systematic arrangement of the genus and discussed briefly the views taken by ANDERSSON and VON SEEMEN. The main purpose of that note was to show that no systematic grouping on natural lines can be attained unless we make use of every taxonomic character.

5. S. POLARIS Wahlenberg, Fl. Lapp. 261. *pl. 13. fig. 1.* 1812; Rydberg in Bull. N.Y. Bot. Gard. 1:264. 1809; Coville in Proc. Wash. Acad. Sci. 3:335. *fig. 27.* 1901.—? *S. herbacea* var. *polaris* Kurtz in Bot. Jahrb. 19:475. 1894.—In America this species is only known from the Alaskan coast of Bering Strait, where it has been collected at Port Clarence and Cape Vancouver. I have seen only the Port Clarence specimens of *Trelease* and *Saunders* (nos. 3387, f., 3385[a], m.), which have been described by COVILLE. They seem to agree with specimens of S. J. ENANDER's Salic. Scand. Exsicc. from Spitzbergen, especially with no. 12 "modificatio foliis subovalibus." The ovaries of no. 3387 are partly glabrate, and I cannot at present say whether the American *S. polaris* is the typical form or not. As to its uncertain systematic position see my preceding remarks.

LUNDSTRÖM (apud KJELLMAN in Nordenskjöld, Vega Exp. Vet. Iakt. 2:21 [Fanerog.-Fl. St. Lawrence-Ön.]. 1883) has described a *S. polaris* f. *subarctica* "foliis tenuioribus, subtus margineque pilis

longis parce adspersis, squamis atris, obtusis; stylo elongato," the type of which was collected by KJELLMAN, July 31 to August 1, 1879, on the northwestern shore of St. Lawrence Island. I cannot interpret this form without having seen the type, and it is not mentioned by COVILLE or RYDBERG. There is, however, a specimen before me collected by *R. L. Shainwald, Jr.*, on Mt. McKinley, 1200 m., August 26, 1903 (fr.; N.), which is very similar to *S. polaris* in every respect, but the fruiting aments measure up to 3 cm. in length and 9 mm. in width. The sessile fruits are 5–6 mm. long, pubescent only toward the apex, and the distinct withered style is a little longer than the stigmas. It looks to me like a new variety of *S. polaris* or a new species.

6. S. UVA-URSI Pursh, Fl. Amer. Sept. **2**:610. 1814; Rydberg in Bull. N.Y. Bot. Gard. **1**:278. 1899; Britton and Brown, Ill. Fl. **1**:601. *fig. 1477*. 1913; Robinson and Fernald, Gray's New Man. 325. *fig. 656*. 1908.—*S. Cutleri* Tuckerman in Amer. Jour. Sci. **45**:36. 1843; And. in Öfv. K. Vet.-Akad. Förh. **15**:132. 1858; in DC., Prodr. **16**^{2}:292. 1868.—*S. Myrsinites* var. *parvifolia* Lange, Consp. Fl. Groenl. **1**:108. 1880; **2**:278. 1887; Fl. Dan. **17**:fasc. 51:13. *pl. 3053*. 1883, non And.—*S. ivigtutiana* Lundström apud Berlin in Öfv. K. Vet.-Akad. Förh. **41**:89. 1884.—A well known willow, of which, however, as I said before, the systematic position is by no means settled. ANDERSSON said, "A *S. arbuscula* recedit foliis minimis parte superiore serrulatis, amentis subterminalibus et capsulis glaberrimis. Longius a *S. retusa* distat. Si ut ferunt auctores americani, flores masculi staminibus tantum singulis praediti sunt, tum affinitas cum *S. coesia* esset, cui etiam rigiditate et glaucescentia foliorum non absimilis, sed folia subserrata et capsulae glabrae." I have very rarely found two stamens in one flower, and I am at present unable to give a precise opinion as to the real relationship of this peculiar species. Its range stretches from New York (Mt. Marcy and Mt. Whiteface), Vermont (Camel's Hump, Mt. Mansfield), New Hampshire (White Mts.), and Maine (Mt. Katahdin) northward to the Gaspé Peninsula, southern and western Newfoundland and the whole coast of Labrador, apparently reaching the northern limit of its range at the southern shore of Baffinsland. Westward it extends through Ungava to the eastern

shores of Hudson Bay. The species has also been reported from southwestern Greenland by DURAND (in Jour. Acad. Nat. Sci. Phil. II. 3:197 [Pl. Kaneanae Groenl.] 1856), but LANGE (Consp. Fl. Groenl. 1:108. 1880) made the following statement: "*S. Uva Ursi* Durand ex descriptione (Pl. Kan.) ad formam nostram 3 [*S. arctophila lejocarpa*] retuli, etsi specimina Kaneana non vidi, quare dijudicare nequeo, an forsan ad veram *S. Uva Ursi* Pursh . . . . pertineant, species, quae tamen a nemine alioquin in Groenlandia lecta esse constat." I am inclined to believe that KANE's specimens represented the true *S. Uva-ursi*, because this species is apparently identical with *S. Myrsinites parvifolia* Lange and *S. ivigtutiana* Lundstr. LANGE said that he did not see specimens of the typical *S. Myrsinites* L. from Greenland, and his var. *parvifolia* seems to be distributed from about the 70th parallel to the very south in Greenland. I have seen one specimen from the Tunugdliarfik Fjord, Kingua, lg. *L. K. Rosenvinge*, August 17, 1888 (fr.; G.), which fully agrees with LANGE's and LUNDSTRÖM's descriptions, and, in my opinion, cannot be separated from *S. Uva-ursi*, the presence of which may be expected in this part of Greenland. The leaves are distinctly glaucous beneath, while they are green and shining on both sides in *S. Myrsinites*. ANDERSSON's var. *labradorica* (1868) from near Oakak on Labrador is scarcely different from the type. He based it on *Hohenacker's* no. 92[e], which I have not yet seen. On the other hand, the forms referred to *S. Uva-ursi* by HOOKER (Fl. Bor.-Am. 2:152. 1839) seem to belong at least partly to *S. arctophila*, but I have as yet seen only a few leaves of *Morrison's* specimen in Herb. Bebb in C. which do not have stomata in the upper epidermis, as is always the case in PURSH's species.

7. S. PEASEI Fernald in Rhodora 19:223. 1917.—This willow is known only from the type locality in New Hampshire, southwest gully of King's Ravine on Mt. Washington, where it was first collected by *Pease* (no. 12091; the type is *Fernald* and *Pease*, no. 16847, fr.; G.). It is certainly a very peculiar species, and needs further observation, the male plant being still unknown. FERNALD's description is, as usual, ample and fitting. I am almost sure that it has to be regarded as of hybrid origin. I visited King's Ravine on September 18, 1918, and I found the willow growing in

about the altitude given by FERNALD on wet cliffs in company with *S. herbacea*. The main part of *S. Peasei* I saw was growing about 15–30 m. below *S. herbacea* on the southern slope of the ravine and covered a rather large area. *S. Uva-ursi* is very common at a somewhat higher level, but I collected plants of it which were growing just above the place where I saw *S. herbacea* and *S. Peasei* close together. Some plants of *S. Peasei* looked much like vigorous *S. herbacea*, while the main part of it lower down at first sight could easily be taken for *S. Uva-ursi*. FERNALD states that *S. Peasei* "finds itself at home on the almost inaccessible wet cliffs," and perhaps he did collect it somewhere else (but apparently not far from where I found it), because this place is by no means "almost inaccessible." Anyone who is a little careful not to start a stone avalanche and is not afraid of some steep climbing can easily visit this locality. Unfortunately the weather became misty and prevented my exploring the southwestern part of the ravine to a greater extent. On the southeastern slopes (toward the Madison Huts) I could not find a trace of either *S. herbacea* or *S. Peasei*, both species seeming to inhabit a very limited area. Under the lens the leaves, above as well as beneath, look as though very finely punctate owing to the presence of stomata. They are not "papillose," as the author says. These stomata are also present in *S. Uva-ursi*, but usually hardly visible except under the microscope.

8. S. HERBACEA L., Sp. Pl. 2:1918. 1753; Lange, Consp. Fl. Groenl. 1:107. 1880; 2:278. 1887; Rydberg in Bull. N.Y. Bot. Gard. 1:277. 1899; Robinson and Fernald, Gray's New Man. 325. *fig. 65*. 1908; Britton and Brown, Ill. Fl. ed. 2. 1:601. *fig. 1478*. 1913.—It has almost exactly the same range as *S. Uva-ursi;* it does not occur, however, in New York and Vermont, and I have not seen specimens from Newfoundland. On the other hand, it is met with on the western shores of Hudson Bay and in western as well as eastern Greenland. It is entirely absent from western North America, but in Europe and Asia its range is even more extensive than that of *S. reticulata*. PURSH (Fl. Am. Sept. 2:617. 1814) cites a specimen of *D. Nelson* from "the northwest coast" which I cannot identify.

9. S. ROTUNDIFOLIA Trautvetter in Nouv. Mem. Soc. Imp. Nat. Mosc. 2:304. *pl. 11* (De Salic. Frig. Kochii). 1832; Andersson in DC., Prodr. 16²:299. 1868; Rydberg in Bull. N.Y. Bot. Gard. 1:276. 1899; Wolf[10] in Izv. S.-Petersburg Liesn. Inst. 5:112. *pl. 38. fig. 15–20. pl. 46. fig. 7–9.* 1900.—*S. polaris* var. *leiocarpa* Chamisso in Linnaea 6:542. 1831.—*S. retusa* var. *rotundifolia* Treviranus ex Trautv. in Nouv. Mem. *l.c.*, pro synon.; Trautvetter in Middendorff, Reise Sib. 1. pt. 2. Bot. Abt. 1:152 (Fl. Boganid. Phaen.) 1847.—*S. rotundifolia α typica* Lundström in Nov. Act. S. Sci. Ups. ser. 3. 1877. 30. *fig. 3.*—*S. leiocarpa* Coville in Proc. Wash. Acad. Sci. 3:338. *pl. 41. fig. 2.* 1901.—"This charming little plant . . . . grows on the islands and both shores of Bering Sea and the Arctic Ocean, and above timber line on the Pacific coast of Alaska eastward to Prince William Sound," and to these localities given by COVILLE is to be added Collinson Point on Camden Bay, where it was collected by *F. Johansen*, June 13, 1914 (no. 39 or 93811 O., m.). The typical form has glabrous ovaries, but two of the specimens before me represent a form with more or less hairy ovaries which I deem best to keep distinct as

f. **pilosiuscula,** f. nov. (ab typo ut videtur nonnisi ovariis partim vel omnino villosis differt). As type may be taken no. 3382 of *Trelease* and *Saunders*, from Hall Island, July 14, 1809 (f.; M.), to which no. 3383 from Matthews Island, July 15 (f.; M.), is to be added. The last specimen has a little longer styles with more or less slender stigmas, thus somewhat resembling *S. stolonifera*, but otherwise not differing from *S. rotundifolia*.

10. S. PHLEBOPHYLLA Andersson in DC., Prodr. 16²:290. 1868; Coville in Proc. Wash. Acad. Sci. 3:336. *fig. 28.* 1901.—*S. anglorum* Chamisso in Linnaea 6:541. 1831, pro parte, quoad specimin. citata; Trautvetter in Act. Hort. Petrop. 6:37. 1879.—*S. buxifolia* Trev. apud Trautv. in Nouv. Mem. Soc. Imp. Nat. Mosc. 2:301. *pl. 10.* 1832,

[10] WOLF, one of our best salicologists, who was curator of the Imperial Institute of Forestry at Petrograd, at least until the outbreak of the war, has made some extremely valuable studies on European and Asiatic willows. Unfortunately his papers are written in Russian, but they are accompanied by excellent sketches. The title of his main paper is (translated) "Materials toward the study of the willows native to European Russia," which appeared in two parts in 1900 in vols. 4 and 5 of the periodical quoted.

non Willd. apud SCHLEICHER.[11]—*S. retusa* Hooker and Arnott, Bot. Beech. Voy. 130. 1832, non L.—*S. arctica β minor* Ledeb., Fl. Ross. 3:619. 1849–51.—*S.* (*retusa*) *phlebophylla* And. in Öfv. K. Vet.-Akad. Förh. 15:131. 1858, pro parte maxima.—*S. arctica β buxifolia* [recte *minor*] Ledeb. ex And. in DC., *l.c.* 290, pro synon.—*S. palaeoneura* Rydbg. in Bull. N.Y. Bot. Gard. 1:267. 1899.—The history of this peculiar willow, which had been thoroughly described by TRAUTVETTER, is well given by COVILLE. ANDERSSON's "species" is always quoted from 1858, but at this time he published the name *phlebophylla* only as a varietal designation, distinguishing 3 forms: *major*, *media*, and *minor*, which he rightly omitted in 1868. I saw a photograph and fragments of all his types preserved in Herb K. The f. *minor* may be represented by a specimen like *Turner's* no. 1293 in part (f.; G.), collected in 1879 on Atka Island. TURNER (Contr. Nat. Hist. Alaska 75. 1886) mentions a *S. rotundifolia* var. *retusa* from Atka Island, "with its heads of cottony catkins peering just above the surface of the other vegetation." I am not quite sure whether he refers to the form before me or not. RYDBERG has mentioned the specimen which I have seen under *S. Dodgeana*, but the leaves do not show the finely impressed veins on the upper surfaces, and the female flowers are very similar to those of *S. phlebophylla*, having, however, a dorsal gland. This form needs further observation, and the species has not yet been recorded so far south in Alaska, where it inhabits the northwestern and northern coast from Norton Sound to Point Barrow. According to COVILLE it was also collected on the Porcupine River by *Turner* in 1891, and SEEMANN (Bot. Voy. Herald 40. 1852) reported it from Pelly's Island at the mouth of the Mackenzie River. On the Siberian coast of Bering Strait *C. Wright* collected it on Arakam (or Kayne) Island. I have also seen a specimen from near Glacier in southeastern Alaska collected in June 1914 by *Mary Milvain* (m., f.; A.).

11. S. DODGEANA Rydbg. in Bull. N.Y. Bot. Gard. 1:277. 1899; Fl. Rocky Mts. 195. 1917; Ball in Coult. and Nels., New Man.

[11] According to SERINGE, Essai Mon. Saules Suisse 54. 1815, the name *S. buxifolia* Willd. was used first by SCHLEICHER, Cat. Sal. 1. 1809, where it is a nomen nudum, as well as in ed. 3 of SCHLEICHER's Cat. Pl. Helv. 25. 1815. This name has to be used, so far as I understand it, for the hybrid *S. glauca*×*S. reticulata;* see BRAND in Koch's Syn. D. Schw. Fl. ed. 3. 3:2357. 1907.

Rocky Mt. Bot. 131. 1909.—This delicate suffruticose species is only known from the type locality, Electric Peak, in the northeast corner of Yellowstone Park (lg. *Rydberg* and *Bessey*, August 18, 1897, f.; N.) and from Sheep Mountain in the Teton Forest Reserve, Wyoming (*F. Tweedy*, no. 292, fr.; N.). RYDBERG calls it "the smallest willow in existence," but there are similar minims among the European (*S. serpyllifolia* Scop.) and Himalayan species (*S. oreophila* var. *secta* And.); see SCHNEIDER in SARGENT, Pl. Wils. 3:146. 1916. The systematic position of *S. Dodgeana* is not yet quite understood; it seems to represent a rather singular type among its American congeners. As to a doubtful pubescent variety, see my remarks under the following species.

12. S. CASCADENSIS Cockerell in Muhlenb. 3:9. 1907; Rydberg, Fl. Rocky Mts. 198. 1917.—*S. tenera* And. in DC., Prodr. 16²:288. 1868, non A. Braun (1850); Rydberg in Bull. N.Y. Bot. Gard. 1:269. 1901; Piper in Contr. U.S.N. Herb. 11:216 (Fl. State Wash.). 1906; Ball in Coult. and Nels., New Man. Rocky Mt. Bot. 136. 1909; in Piper and Beattie, Fl. Northwest Coast 117. 1915; Jepson, Fl. Calif. 344. 1909, pro parte.—*S. phlebophylla* Watson in U.S. Geol. Sur. Expl. 40th parallel King's Rep. 5:Bot. 326. 1871, pro parte, non And.—*S. arctica* var. *petraea* Bebb in Watson, Bot. Calif. 2:90. 1879, pro parte, non And.; Ball in Trans. Acad. St. Louis 9:89. 1899, pro parte.—*S. Brownii* var. *petraea* Bebb in BOT. GAZ. 16:107. 1891, pro parte.—*S. Brownii* var. *tenera* Jones, The Willow Fam. 19. 1908.—This species has always been regarded as most closely related to *S. petrophila*, and BALL (1899) mentioned it in the synonymy only as "a narrow-leaved form," while he (1909) says "perhaps only a variety of the preceding" (*S. petrophila*). By a close study of the material before me I have the impression, however, that it possibly might have a more intimate affinity to *S. phlebophylla*. Both BALL and RYDBERG distinguish *S. cascadensis* from *S. petrophila* only by the smaller size of the leaves and the few-flowered aments, and neither author mentions the fact that the old leaves are more or less persistent, a character not observed by me in *S. petrophila*. The leaves of *S. cascadensis* are occasionally up to 18 mm. long, and in male specimens, like no. 1074 of *Merrill* and *Wilcox* from the Teton Mountains, Wyoming, the catkins bear

more than 20 flowers, and some fruiting aments before me measure up to 2.5:0.9 cm. The systematic position of the species needs further study, however, and it is interesting to note the singular view expressed by ANDERSSON himself as to the relationship of his *S. tenera:* "Memorabilis forma a nostris *S. retusa*, *reticulata*, et *arbuscula* quasi composita. *S. retusae* similis: foliis lingulatis, vix $\frac{3}{4}$ poll. longis, supra medium 2 lin. latis, parallelo-nervosis, integerrimis; *S. reticulatae:* capsulis sessilibus, dense lanatis, pusillis; et *S. arbusculae:* amentis ramulos laterales terminalibus."

The type of *S. cascadensis* was collected by LYALL in 1860 on the "eastern summits of Cascade Mountains" (f.; K.), and LYALL's specimens were sent out from Kew under the name *S. phlebophylla;* therefore BEBB (in BOT. GAZ. 16:107. 1891) accused ANDERSSON rather unjustly of the use of this name for LYALL's plant. There is no proof that ANDERSSON himself applied his own name *phlebophylla* to this form which he (1868) made the type of *S. tenera.* The writing on the labels of LYALL's specimens is not in ANDERSSON's hand. The specimen collected by WATSON in the Uinta Mountains, Utah (no. 1101, f.; G.), which the collector referred to *S. phlebophylla* because it seemed to match perfectly LYALL's plant, is of peculiar interest; it looks to me more like a pubescent form of *S. Dodgeana* than anything else, but I do not venture at present to pass final judgment upon it.

Sect. III. OVALIFOLIAE Rydberg in Bull. N.Y. Bot. Gard. 1:274. 1899, pro parte, sed sensu emend.—Sect. ARCTICAE Rydbg., *l.c.* 263, pro parte, non And.; Ball in Coult. and Nels., New Man. Rocky Mt. Bot. 135. 1909, pro parte.—Sect. DIPLODICTYAE Schneider in Sargent, Pl. Wils. 3:136. 1916.—See my first note (BOT. GAZ. 66:117. 1918). To this section belong the following species I have dealt with (*l.c.*): 13. *S. petrophila* Rydbg., 14. *S. anglorum* Cham., 15. *S. arctica* Pall., 16. *S. ovalifolia* Trvt., 17. *S. stolonifera* Cov., and 18. *S. groenlandica* Ldstr. I wish to add some notes to the following species.

15. S. ARCTICA Pall.—Since I wrote the first article I have seen specimens (*Evans*, no. 439, partim; W.) from Kodiak which represent TRAUTVETTER's *S. diplodictya* mentioned *l.c.* 123. It is nothing but a mere form with "foliis utrinque concoloribus."

17. S. STOLONIFERA Coville.—I mentioned (*l.c.* 137) a new f. *subpilosa* of which as type may be taken B. E. FERNOW's specimen from Glacier Bay, Point Gustavus, June 10, 1899 (Cor.). There are specimens before me from the same bay, Muir Glacier, collected between June 8 and 12, 1899, by *F. V. Coville* and *T. H. Kearney* (no. 621, f.; W.). COVILLE is of the opinion that they, at least partly, represent a hybrid between *S. arctica* and *S. stolonifera*. One of the sheets (no. 373447, W.) looks indeed like such a hybrid; the leaves seem not to possess stomata in the upper epidermis and are partly larger, while the flowering aments measure up to 3:1 cm. The other specimen (of sheet no. 376920), however, can hardly be separated from *S. stolonifera* except by the tomentose ovaries. The leaves have numerous stomata in the upper epidermis, are of the usual size and shape, and the aments are not larger than in typical *stolonifera*. This specimen looks to me more like f. *subpilosa* than like a hybrid. It may be, however, that we have to take all the specimens with more or less pubescent ovaries and fruits for those of hybrid origin, but this does not seem likely to me.

18. S. ARCTOPHILA Cock.—Professor T. D. A. COCKERELL kindly informed me that he had published in the last edition of HELLER's Cat. N. Am. Pl. p. 89, 1910, this new name for *S. groenlandica* Lundström but not of Heer (Fl. Foss. Arct. 1:101. *pl. 4. figs. 8–10.* 1868). I was not aware of this fact, neither had I seen the sheets of this edition of HELLER's Catalogue. Besides, COCKERELL's name is not found in the card index published by the Gray Herbarium. I accept this new name, but there is no definite statement in the international rules as to the priority of names of paleontological plants. Owing to this change I have to propose the following new combination, *S. arctophila* var. **lejocarpa** for *S. groenlandica* var. *lejocarpa* Lange (*l.c.* 141).

18a. **S. hudsonensis,** spec. nov.—*S. fullertonensis*×*S. groenlandica* Schneider in BOT. GAZ. 66:342. 1918.—Frutex prostratus habitu *S. arctophilae,* ramis pl.m. subterraneis radicantibus ad 5 mm. crassis, ramulis elongatis repentibus glabris ut in *S. arctophila* coloratis, gemmis foliisque ut l.c. a me descriptis sed gemmis interdum ad 9 mm. longis et foliis angustioribus acutioribus ad 3.5:1.7 cm. latioribus ad 3.5:2 cm. magnis superne vulgo haud stomatiferis

maturis glabris vel utrinque sparse pilosis; amentis tantum fructiferis visis 2: 1.3 ad 4: 1 cm. magnis ramulos foliatos ad 4 cm. longos terminantibus; fructibus ad 9 mm. longis pedicello brevissimo vel glandula vulgo distincte breviore excluso.

Besides the specimens mentioned (*l.c.* 342), I examined the following: Hudson Bay: 50 miles south of Cape Eskimo, August 5, 1900, *E. A.* and *A. E. Preble* (no. 43, fr., type, 46, st.; W.); 25 miles south of Cape Eskimo, August 12, 1900, same coll. (nos. 54, 57, fr.; W.).

After having seen the material collected by the PREBLES I think it best to propose a new name for this interesting form, which after all seems to represent a new species closely related to *S. arctophila*, from which it chiefly differs in the shorter pedicels and the more elongated gland. Judging by the flowers alone, one might be inclined to take it for a form of *S. anglorum*, but the leaves are mostly without any trace of stomata in the upper epidermis, and their color and texture are more like in *S. arctophila*. Some specimens cited in my second note may represent hybrids between this species and *S. fullertonensis*. Unfortunately I have not yet seen young female or male flowers, and further investigation is needed to elucidate the real affinity of this form, which seems to be fairly common along the western shores of Hudson Bay from James Bay to Cape Fullerton and also on the islands in the western part of Hudson Strait.

Sect. IV. GLAUCAE E. Fries, Syllog. Pl. Nov. 2:36. 1828, pro parte.—Sect. ARCTICAE Rydbg. in Bull. *l.c.* pro parte; Ball in Coult. and Nels, *l.c.*, pro parte. For further synonymy see SCHNEIDER in Sargent *l.c.* 147. 1916.—In my second paper (BOT. GAZ. 66:318. 1918) I have already explained the differences between this section and the ARCTICAE, and there I have also discussed the following species, which are to be referred to this section: 19. *S. fullertonensis* Schn., 20. *S. niphoclada* Rydbg., 21. *S. chlorolepis* Fern., 22. *S. brachycarpa* Nutt., 23. *S. pseudolapponum* v. Seem., 24. *S. desertorum* Rich., 25. *S. glauca* L., 26. *S. cordifolia* Pursh, 27. *S. anamesa* Schn., and 28. *S. lingulata* And.

I wish to add the following remarks, because I had the opportunity to study some very interesting material of Herb. W., and I

desire to express my gratitude to the curator of the U.S. Nat. Herbarium.

20. S. NIPHOCLADA Rydbg.—Having seen a co-type and other material of this species from Herb. W., I wish to correct my statements (*l.c.* 339) as follows: The co-type collected by *Miss E. Taylor* has ripe fruits which measure up to 7 mm. in length and show a distinct pedicel (about 1.3 mm. long) which is distinctly longer than the bifid gland. The male type described by COVILLE (*Funston*, no. 185) has short aments measuring about 10:4–5 mm. and loosely flowered at the base, but there is a specimen collected by *F. C. Schrader* on the John River in northern Alaska, July 10, 1901, of which the female part well agrees with the type of *S. niphoclada*, while the male aments measure up to 22:4 mm., being very slender and loosely flowered. In FUNSTON'S specimens the male flowers are younger, but I hardly believe that they could grow to the size of those of SCHRADER'S plant. Otherwise the flowers are identical, having glabrous filaments and ventral glands of a similar shape. SCHRADER, however, collected another male specimen on Anaktuvuk River (erroneously spelled Ansktoobah River on the label) August 5, 1901, of which the aments are like those of the John River plant, but the filaments are somewhat hairy at base. Otherwise I cannot separate SCHRADER'S plants, and I believe that the pubescence of the filaments which mostly can be taken for a constant character may not be of reliable taxonomic value in this case. We need, of course, a better acquaintance with all the willows of this region to decide the question whether or not the absence or presence of a pubescence on the filaments is a really important character. A fruiting specimen collected by SCHRADER at the same place and date as the last mentioned male one seems to me inseparable from *S. niphoclada*, but the ripe fruits measure up to 8 mm. in length, are almost sessile, and of a more ovoid-conical shape than in the type.

The specimen mentioned (*l.c.* 339) from Fort Churchill, Hudson Bay, collected by *E. A.* and *A. E. Preble*, is really no. 41, not no. 26 (as given by me and by COVILLE), according to a note by E. A. PREBLE on the sheet (no. 385093) in Herb. W. From the same place the same collectors brought a male specimen (no. 33; W.) which I refer to *S. brachycarpa*, which had been collected there

also by *F. M. Macoun* (*l.c.* 337). PREBLE'S no. 41 may be more closely connected with *S. brachycarpa* than with *S. niphoclada*, but the shape of most of the larger leaves is more elongate-lanceolate in no. 41, where the largest leaves measure up to 4.5: 1 cm., while those of MACOUN'S female plant are shorter and broader, and its pubescence is more like in typical *S. brachycarpa*. In no. 41 the young twigs bear a very thick villose pubescence of long soft hairs, which is far more conspicuous than the tomentum of the forms of *S. niphoclada* from the west. After all, I am inclined to believe that no. 41 may represent a form of *S. brachycarpa* comparable to f. *poliophylla* of *S. glauca acutifolia*. We must not forget, however, that we hardly can elucidate those forms without a full understanding of the true *S. desertorum* (see *l.c.* 331).

I have thought it best to treat those forms at a considerable length, because we know so little of the willows of the Northwest Territories, and I wish to give an impulse to their closer investigation in the field by future collectors.

21. S. CHLOROLEPIS Fern.—I have described (*l.c.* 339) the var. *antimima*, which looks rather intermediate between the type and *S. brachycarpa*, and which on Mt. Albert seems to be connected by hybrids with the latter. There is a small male specimen in Herb. N. collected by *A. P. Low*, north of Cape Jones on the eastern coast of Hudson Bay, July 16, 1898 (no. 63272 O.). It agrees well with *S. brachycarpa*, and has hairy filaments, but I found some stomata in the upper leaf epidermis. We need much more copious material (male and female) from this locality to judge the form properly.

25. S. GLAUCA var. ACUTIFOLIA (Hook.) Schn.[12]—Having received, as already stated, very interesting material from Herb. W., I wish to add the following remarks.[13] There are before me several

[12] I stated (*l.c.* 321) that 2 *S. villosa* had been published before HOOKER described the present form under this name, but there is still a much older *S. villosa* Hoffmann (Obs. Bot. 15. 1787) which is not registered in the Kew Index, nor can I find this name mentioned by KOCH, FRIES, WIMMER, ANDERSSON, or V. SEEMEN. It was sent from Sweden by THUNBERG.

[13] In the note (*l.c.* 321) I made an entirely wrong statement in regard to PURSH'S Canadian collections. Relying on facts given by HARSHBERGER, which he in his turn took from an article in BOT. GAZ. 7:142. 1882, I said that PURSH did not collect in Canada. Professor N. L. FERNALD kindly directed my attention to PENHALLOW'S

specimens collected at Great Slave Lake, Mackenzie, which, in my opinion, are most closely related to var. *acutifolia*, but at least some of them seem to represent a very villose form of it, for which I propose the name: S. GLAUCA var. ACUTIFOLIA f. **poliophylla,**[14] forma nov.—A typo nonnisi differre videtur ramis annotinis densius villoso-lanuginosis etiam vetustioribus tomento lanuginoso pl.m. obtectis, foliis superne pl.m. laxe adpresse sericeo-lanuginosis subtus villo densissimo molli pl.m. adpresso albo vestitis. The type is Great Slave Lake, Fort Rae, July 28, 1901, *E. A.* and *A. E. Preble* (no. 139, fr.; W.; folia inferiora elliptica vel oblongo-elliptica, utrinque acuta, superiora magis ovato-elliptica apice acutiora, maxima ad 5:2.2 cm. magna; amenta fructifera pedunculo foliato ad 3 cm. longo excluso ad 5:1.3 cm. magna; fructus e basi rhomboideo ovoideo-conici, ad 9 mm. longi pedicello ad 1.5 mm. longo glandulam subduplo superante excluso).

The following specimens seem to me rather intermediate between f. *poliophylla* and typical var. *acutifolia:* Great Slave Lake: Fort Resolution, July 14, 1901, *E. A.* and *A. E. Preble* (no. 141, fr.; W.; forma minus dense quam no. 139 villosa); June 21, 1903, *E. A. Preble* (no. 194, f.; W.; eadem forma ut videtur ac praecedens sed juvenilis); Fort Good Hope, on the Mackenzie River, June 23, 1904, *E. A. Preble* (nos. 330, f. et fr. anni praeteriti, 332, f.; W.; forma a cl. RYDBERG ad *S. niphocladam* relata foliis floribus juvenilibus; sed folia distincte petiolata et fructus adulti magni). There are two other male specimens of E. A. PREBLE from Fort Resolution, June

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"Review of Canadian Botany" (Trans. Roy. Soc. Can. III. 4:4. 1897), where he expressly states that "with the exception of his immediate predecessors no botanist had accomplished more than PURSH to make the vegetation of Canada known." When JAMES published the manuscript of PURSH'S traveling journal he did "not deal with that part of PURSH'S work which was continued into and ended in Canada." "He made extensive collections chiefly through the province of Quebec, but all the material thus accumulated was subsequently destroyed by fire."

Regarding Lord SELKIRK, I have been informed by J. C. NELSON that "Lord Selkirk's Exp." probably refers to THOMAS DUNDAS, Fifth Earl of Selkirk, who (according to JOHNSON'S N. Univ. Cycl. 4:175. 1878) "spent several of the later years of his life in promoting emigration to the Red River of the North, British America." I have not yet been able to consult the tracts which he has published on emigration to those parts of Canada.

[14] Derived from πολιός, with white hair.

21, 1903 (nos. 196, 198; W.), which RYDBERG regards as "near *niphoclada* but with much broader and shorter leaves." The young leaves do not differ from those of the female specimen no. 194 previously cited, but the young male aments are very short, not exceeding 1 cm. in length and 0.5 cm. in thickness. Otherwise the flowers agree with those of var. *acutifolia*. If the size of the male aments should prove a reliable character, and if the male plant should belong to the same form as the female no. 194, this form might prove to be a new one more closely related to *S. cordifolia*. See also my remarks under *S. niphoclada* as to the size of male aments.

There is a male specimen collected by *Seton* and *Pringle*, July 19, 1907, near Caribou Island, Great Slave Lake (no. 43 [78305 O.]), which has been named *S. atra* Rydbg. by BALL. At first sight it resembles a great deal PREBLE'S nos. 196 and 198 mentioned above, but the leaves, which seem to be almost fully grown, measure only up to 3.6:1.6 cm. The flowers of the one small catkin (2.3:0.9 cm.) I could examine show no trace of a dorsal gland, and the hairy filaments are connected for $\frac{1}{3}$ to $\frac{1}{2}$ of their length. So far this form remains rather doubtful.

26. S. CORDIFOLIA Pursh.—The male syntype of *S. labradorica* mentioned (*l.c.* 345) bears the number 21, not 31, of WAGHORNE.

27. S. ANAMESA Schn.—In his Fl. Europ. 21:157. 1890, GANDOGER has described under *S. glauca* the following 4 new subspecies from Greenland: *S. eskimorum* (type: *Petersen*, Julianehaab); *S. groenlandica*, non Heer, nec Lundström (type: *Rink*, Godthaab); *S. platycarpa* (type: *J. Vahl*, Ikilok), and (*l.c.* 158) *S. Vahlii* (type: *J. Vahl*, Ikilok). Judging by the characters given in the key, all these names refer to a form having "folia anguste lineari-oblonga, 5–12 mm. lata," which are "supra semper canescentia et villosa vel tomentosa" in the last two forms, while they are "supra glabra aut glabrescentia et viridia" in the first two. Not having seen the types, I am unable to say to which species these forms really belong, but I presume they may be referable to what I have called *S. anamesa*. In giving binary names to those forms GANDOGER did the same as ANDERSSON did in several instances in his monograph. I believe, however, that these binomials cannot be regarded as

proper species, and therefore I do not think it advisable to take up one of GANDOGER's names for *S. anamesa*.

V. SPECIES SECTIONIS INCERTAE. The proper systematic position of the following species is still doubtful to me, and most of them are so little known that it is impossible to even express an idea as to their affinities.

29. S. CHAMISSONIS And. in DC., Prodr. 16²:290. 1868; Lundström apud Kjellman in Nordenskiöld, Vega Exp. Arbet. 2:21 (Fanerogfl. St. Lawrence Ön). 1883; Coville in Proc. Wash. Acad. Sci. 3:325. *fig. 23.* 1901.—*S. myrsinites* Chamisso in Linnaea 6:540. 1831, non L.—The type of this rare but apparently well marked species was collected by CHAMISSO and ESCHSCHOLTZ in 1816 at St. Lawrence Bay; elsewhere it is only known from St. Lawrence Island and Port Clarence in Alaska.[15]

30. S. GLACIALIS And. in Öfv. K. Vet.-Akad. Förh. 15:131. 1858; in DC., Prodr. 16²:300. 1868; Coville in Proc. Wash. Acad. Sci. 3:329. *fig. 24.* 1901; Rydberg in Bull. N.Y. Bot. Gard. 1:262. 1899.—*S. Uva-ursi* Seemann, Bot. Voy. Herald 40. 1852, pro parte. —As COVILLE has already stated, "this species is known only from the type specimen collected by Lieutenant *W. J. G. Pullen* in 1849, on the Arctic seacoast between Point Barrow and the Mackenzie River, and from specimens collected at Point Barrow by *John Murdoch* in 1882–3." I have been able to examine a photograph and fragments of the type (*Pullen*, no. 155, f., fr. im.; K), and I agree with ANDERSSON that it is a forma *pusilla* of *S. ovalifolia*. The style is very short but not wanting, and the stigmas are relatively long; the pedicel is also distinct and in fruit about ⅓ longer than the gland. It resembles the pubescent form of *S. ovalifolia*, which is only somewhat more vigorous, and shows a more distinct (but short) style. ANDERSSON describes the leaves of the type as

[15] After the manuscript of this note had gone to the printer, I found on a sheet with *S. amplifolia* of Herb. Cor. a male specimen of *S. Chamissonis* collected by B.E. on St. Lawrence Island, July 13, 1899, which had been seen by COVILLE but is not enumerated by him in 1901. Had I seen it before I finished my manuscript I would not have included this species in the present key because, judging by the male flowers, it seems to belong to sect. COMMUTATAE, with which I hope to deal later. The male flowers possess only one gland, and the fine and close acute serration of the leaves easily distinguishes this species from all the other willows treated in this paper.

"integerrima," while most of them in the specimen before me show a distinct but fine glandular denticulation in the lower half. Those of the type are entire, with the exception of a few minute teeth at the base of some leaves. They lack stomata in the upper epidermis. If I had seen the type before I finished the manuscript of this article, I would have placed *S. glacialis* next to *S. ovalifolia*, but as long as the male flowers are unknown the true affinity remains unknown.

31. S. VENUSTA And. in DC., Prodr. 16²:288. 1868, non Host 1828, from Sitka, of which, according to COVILLE, the description "suggests that the plant may prove to be a form of *Salix reticulata* grown in a shaded situation," can only be judged by an examination of the type material, which I have not yet had the opportunity to see.

32. S. OBCORDATA And. in DC., Prodr. 16²:291. 1868, which, like the preceding, came from Sitka, is even more obscure than that, and may prove to be another *S. reticulata* form or a hybrid of it with some other species. ANDERSSON himself placed it without number as *S. obcordata* between *S. ovalifolia* and *S. furcata*, but he says nothing about its relationship to these species, only mentioning its resemblance to *S. venusta* and *S. reticulata* in his more than meager description.

I hope that my notes may prove useful to other students of American willows, and I shall be most thankful to anyone who can correct any mistake I have made or furnish me with good material or information of these or other American willows. There are, of course, quite a number of specimens before me which I have not yet been able to elucidate. Among them are many which I suspect of being of hybrid origin, but I do not intend to deal with the hybrids until I have gained a more thorough understanding of all the American forms of this difficult genus.

ARNOLD ARBORETUM
JAMAICA PLAIN, MASS.

MORPHOLOGY OF THE GENUS ACTINOMYCES. I[1]

CHARLES DRECHSLER

While the genus *Actinomyces* has received a large measure of attention in its relations to soil biology and to human, animal, and plant pathology, the natural affinities of the congeneric organisms that it has been customary to include in the group have been the subject of diverse opinions. Under a variety of synonyms, among which *Cladothrix*, *Nocardia*, *Discomyces*, and *Oospora* have been used nearly as frequently as *Actinomyces*, the group has been placed with the bacteria, with the Hyphomycetes, or assigned to an intermediate position. In the earlier publications on the ray fungus, including the papers by BOSTROEM (1)[2], and by WOLFF and ISRAEL (24), this organism was referred to the pleomorphic bacteria. The belief was seriously entertained that cocci, bacteria, and spirilla were produced by the plant, and in such regular succession that a number of investigators were led to draw up detailed ontogenetic schemes of considerable complexity. It is frequently not easy to determine the exact nature of the structures that were interpreted as pleomorphic stages. There are plenty of indications that contaminating bacteria were often present as secondary invaders; but more frequently aërial spores, segments of spiral or sinuous hyphae, and degenerative bodies of metachromatic substance were mistaken for schizomycetous types of nearly every description.

More recently *Actinomyces* has been frequently associated with the tubercle and diphtheria organisms on the assumption that they may represent a transition between the Hyphomycetes and the true Schizomycetes. A family of Actinomycetes has thus been erected as a natural group from these diverse components, united chiefly by resemblances in their staining reactions, a usual or an occasional filamentous habit, and the development of clavate elements in the animal body. It has been supposed by adherents of such a taxonomic disposition that either a progressive phylogeny

[1] Contribution from the Cryptogamic Laboratories of Harvard University, no. 83.

[2] The bibliography will appear at the end of Part II, which will be printed in February.

has occurred in the family, with *Actinomyces* at the head of a transition series developing increasingly well marked fungoid characteristics, or else that *Actinomyces* is the probable progenitor of the groups *Corynebacterium* and *Mycobacterium* by degenerative reduction.

The view that the ray fungus represents an organism with hyphomycetous affinities was advanced early by HARZ (7) and DEBARY (2). These authors regarded as conidia the clavate elements of the actinomycotic lesion, which BOSTROEM's studies later properly degraded to the rank of degenerative structures. SAUVAGEAU and RADAIS (20), DOMEC (3), THAXTER (22), GASPERINI (4), and others placed a number of congeneric forms among the Hyphomycetes on account of their production of aërial spores. It may be mentioned in this connection that an examination of a considerable number of species has convinced the writer that this disposition is the only one which is in harmony with the morphological conditions represented in the genus.

The material used in these studies, with the exception of authentic cultures of the species described by WAKSMAN and CURTIS (23), and of a number of organisms isolated by H. J. CONN from soil collected near Geneva, New York, was largely obtained from soil collected in Cambridge, Massachusetts. By the use of the dilution method more than 1000 plants belonging to the genus were isolated from this source; and of these about 300, representing probably more than 100 species, were selected for morphological examination. Approximately 400 additional individuals were derived from soil collected in Porto Rico, Cuba, Panama, Montana, Wisconsin, and Kansas, as well as from outdoor air, tap water, horse manure; and gross cultures of dung, dead leaves, and other vegetable matter. The potato scab organism was obtained from Mr. M. SHAPAVALOV, who had isolated it from a diseased tuber, and experimentally established its pathogenicity.

The morphology of the vegetative thallus of *Actinomyces*, apart from its astonishing minuteness, the diameter of the filaments ranging commonly from 0.5 to 1.2 μ, presents no features unusual among the fungi. In most species the mycelium is generally sparsely and irregularly septate; and although in other forms trans-

verse walls may appear with somewhat greater frequency, there are none in which septation approaches any pronounced degree of regularity or closeness. Ramifications are abundant, and the branching is altogether of the "true" type. Macé (15), who first carefully observed the formation of branches, found it to proceed by the elongation of lateral buds arising some distance back from the growing point of an axial filament, the branch thus produced giving rise similarly to secondary branches by lateral proliferation. Lachner-Sandoval (12) confirmed Macé, designating the process as monopodial and denying the existence of true dichotomy in the genus, which had been affirmed by previous investigators. Later Neukirch (18) reported that the branching in *Actinomyces ochroleuceus* was occasionally of the nature of a true dichotomy. From an examination of very young mycelium (fig. 3)[3] it is apparent that, at least in stages following the germination of the spore, filaments are not infrequently terminated by two elements too closely similar in size and angular relationships to be distinguished as bud and axial tip. The branching in such cases must be regarded as dichotomous, although all gradations toward the prevailing well defined monopody may be found. It seems reasonable to suppose, however, that the distinction is one of convenience, not implying any fundamental difference in manner of development.

The cytological structure of *Actinomyces* is equally devoid of bacterial characteristics. The branches forming the periphery of the actively growing pellicle, or the young sporogenous branches attached at intervals to the superficial mycelium, are filled with dense protoplasm, which, with haematoxylin, takes a deep homogeneous stain. Further toward the origin of the hyphae the contents become more attenuated, and vacuoles appear, increasing in number and size until they occupy the larger portion of the filaments. When individual vacuoles become excessively large and extend through a considerable length of filament, the cytoplasm is in a large measure confined to a peripheral layer, a condition which led Neukirch to distinguish a thin, strongly refringent "Aussenplasma" and a less refringent "Innenplasma."

[3] The plates will appear in connection with the second part, to be published in the following number.

The presence of large vacuoles is commonly associated with local distentions of the filament wall. These may occur with such regularity in the degenerate mycelium of some species as to suggest the appearance of *Leptomitus*, each swollen segment being largely occupied by a single elliptical vacuole, separated from the vacuole in the adjacent distention by a protoplasmic partition at the constriction (figs. 47, 48, 106). In other species and quite generally in the nutritive mycelium of all forms, that is, the mycelium immersed in the substratum, no marked regularity in the alternation of inflated portions and constrictions is observable; but pronounced deviations in the diameter of filaments may occur with more or less variable frequency.

A great deal of importance has been attributed by some writers to a variety of abnormalities and products of degenerative changes occurring in the thallus of *Actinomyces*. In the earlier literature on the ray fungus, especially in the publications of ISRAEL (8), JOHNE (9), and MACFADYEAN (16), bodies described as "micrococci," "cocci," or "coccus-like granules" were given minute attention, and assigned an important rôle in the complex ontogeny ascribed to the parasite, then supposed to belong to the pleomorphic bacteria. WOLFF and ISRAEL (24), whose photomicrographs of these bodies leave no reasonable doubt about their identity with structures very frequently observed by the writer (figs. 15, 31, 32, 42, 91), confused them with the spores reported by other authors; and as the structures did not possess the heat resistance common to spores of bacteria, these investigators were inclined to question the production of spores by *Actinomyces*. Since the organism used by WOLFF and ISRAEL was constantly sterile, their conclusion concerning it was undoubtedly correct. BOSTROEM, who experimented with a sporiferous form, did not succeed in avoiding the same confusion, and refers indiscriminately to the unicellular products formed from aërial hyphae, and to the spherical endogenous granules, as "spores."

Round granules, deeply stained in the living filament by very dilute methylene blue, were studied later by NEUKIRCH. He noted in them a variable size, a method of multiplication, and an orientation related to the regions of growth in the thallus. These

observations led him to believe that the structures represented nuclei. SCHÜTZE (21), who applied NEUKIRCH'S method of staining, designated the bodies as metachromatic granules. After an examination of their occurrence in the aërial mycelium of a considerable number of species, such an interpretation seems, in the opinion of the writer, to offer the greater degree of plausibility.

The metachromatic material is easily distinguished by a powerful affinity for most of the stains ordinarily employed in laboratories. In material fixed in alcohol, and treated with Delafield's hematoxylin, it retains a nearly opaque stain after all other structures have been completely decolorized. Indications of its presence in the tips of growing filaments, or in sporogenous branches, in general are very infrequent. Some distance toward the origin of the hyphae, associated with a more attenuated or vacuolated protoplasm, the material makes its appearance in the form of rather minute granules widely separated from one another. As the filament is followed still farther back, the granules increase in size and frequency; often their arrangement is one of much regularity, the individual spherical bodies being of nearly equal size, exactly filling the lumen of the filament, and separated by nearly equal spaces (fig. 42). In other cases the granules seem to coalesce and occupy entire segments of hyphae (fig. 32); and in a few species extensive portions of mycelium were frequently found entirely filled with long unbroken masses of metachromatic substance. It is this property of coalescence of smaller granules, to form incomparably larger masses, bearing out the similarity in appearance to a homogeneous liquid with a relatively high surface tension, that makes it difficult to believe that we are dealing here with anything relating to spores or to nuclei.

The function of the metachromatic material in the *Actinomyces* thallus cannot be ascertained with certainty. A number of views have been advanced regarding the rôle of metachromatic substance in the cell, none of which has gained universal acceptance. The best explanation, in the opinion of the writer, seems to be that it represents an occluded waste product. While its presence in small or moderate quantities in the sterile hyphae bearing the sporogenous branchlets is probably more or less normal, its abundant

occurrence here, as everywhere else, is an indication of advanced degeneration. In the more mature mycelium of *Actinomyces* VIII (fig. 47) metachromatic granules are usually very conspicuous, often occupying most of the space in the narrowed constrictions between the large vacuoles in the highly inflated mycelial segments. The appearance of such a thallus is not in the least suggestive of the structure of bacteria, and indicates that the resemblance between *Actinomyces* and the true Schizomycetes in the consistency of protoplasm, emphasized by some writers as an important phylogenetic connection, has been unduly overestimated.

While the sterile filaments in the nutrient and in the aërial mycelium are relatively uniform in structure throughout the genus, the sporogenous apparatus of many species exhibits a large degree of morphological individuality. This diversity has not usually been recognized by writers, and has undoubtedly been responsible for a portion of the controversy that has arisen, particularly with regard to the method of spore formation. LACHNER-SANDOVAL (12), from a study of *Actinomyces albido-flava*, distinguished two kinds of propagative bodies: (1) fragmentation spores appearing as spherical to cylindrical segments in old hyphae, formed by a contraction of the protoplasm; and (2) segmentation spores developed by a septation of the tips of aërial filaments. Segmentation was usually found to involve only lateral branches coming from aërial hyphae, but in submerged growths it frequently extended also to the main filament, leading to the development of a dendroidic system of spore chains. LACHNER-SANDOVAL'S figures of these formations, however, are much less striking than might be expected from the description in the text, and do not convey the impression of ramifications approaching treelike proportions.

NEUKIRCH identified the segmentation of LACHNER-SANDOVAL with oidium-spore formation among the fungi, and abandoned the use of a specific term. He vigorously disputed the development of aërial spores by a septation of the mycelium. According to his account the spores are formed as the result of successive contractions of protoplasm until approximately isodiametric portions are separated by regularly alternating empty spaces. This process he identified with the fragmentation of MACÉ, localizing it in a differ-

ent region from LACHNER-SANDOVAL, and properly relegating the fragmentation of the latter to the category of degenerative changes.

GILBERT (5) found some lateral branches to begin the process of forming spores by becoming differentiated into highly refractive and weakly refractive portions. Constrictions later appear, unassociated with visible changes inside the filament, and soon the spores are completely cut off. GILBERT designated the process as segmentation, following LACHNER-SANDOVAL, who, however, had actually observed septa appearing more or less simultaneously with the constrictions, their appearance being followed by the enlargement and rounding up of the segments to form spores.

MIEHE (17), in his study of *Actinomyces thermophilus*, only incidentally examined the mode of sporulation. He believed spores were produced singly on very short stalks attached laterally to the main hyphae, or possibly by successive contractions in chains. In either case conidia were produced, not by the segmentation of a completed filament, but by the development of a structure which at no time constituted a cylindrical, continuous hypha. This account, in general, bears strong resemblances to the later description by SCHÜTZE (21) of *Actinomyces monosporus*, a form in which the spores are borne singly on delicate stalks in racemose arrangement on a thicker axial filament. It might well be questioned, however, whether forms like this, which depart so widely from the main morphological trend of *Actinomyces*, are properly to be assigned to this genus, even if allowance is made for much liberality in the definition of hyphomycetous form-genera.

The same criticism, however, cannot be extended to the condition described by SCHÜTZE in his strain of *Actinomyces thermophilus*. In his account of this species its author strongly defended NEUKIRCH'S position that the mode of sporulation was one of fragmentation. However, while NEUKIRCH found long filaments converted into spore chains by successions of protoplasmic contractions, the long portions finally becoming resolved into ultimate spores, SCHÜTZE found that only short terminal portions or short lateral branches yielding about 5 spores were involved. According to NEUKIRCH, the slightly refractive spaces between the masses of protoplasm that later develop into spores are entirely empty, and

the intervening portions of filament wall merely collapse as the spores mature. SCHÜTZE believed that these intervals were filled with attenuated protoplasm, and that by their constriction the spores were delimited without the evacuation of portions of hyphal wall. The spore of NEUKIRCH, consequently, is a structure possessing its own spore wall, enveloped, except at its ends, by the remnants of the old filament wall; that of SCHÜTZE, on the other hand, is without a separate spore wall, the filament wall constituting the only membrane present, and forming a spherical shell everywhere inclosing the protoplasm.

NEUKIRCH gave much attention to certain structures he designated as oidium-spores. They developed in submerged growths, the transformation of the filament consisting only in more or less close septation, followed by a slight swelling of the segments. Under suitable conditions filaments grew out from them, an occurrence NEUKIRCH regarded as germination. "Aussenplasma" and "Innenplasma," in his opinion, were sharply defined, but a spore wall was absent. The elements did not exceed the filaments in resistance either to heat or desiccation. NEUKIRCH believed their function to be the dissemination of the fungus in liquid media.

LACHNER-SANDOVAL seems to have seen the same structures and regarded them as segmentation spores that had developed in the submerged condition. GILBERT, SCHÜTZE, and KRAINSKY (10) record their failure to find these bodies without, however, denying their existence. According to SCHÜTZE and GASPERINI, sporulation may occur in hyphae which are not truly air hyphae.

It seems questionable whether any desirable end is served by calling NEUKIRCH'S elements spores at all. To apply the term to structures with so little individuality, even though a sort of promiscuous viability may be attributed to them, is approaching very close to the point where all bodies not filaments of uniform thickness are to be regarded as spores. Certainly the distended elements in old mycelium of *Actinomyces* VIII (figs. 47, 48), which represent enlargements of axial filaments developed gradually in the course of time at the junctures with moderately complex systems of sporogenous hyphae, frequently have an equal or greater resemblance to reproductive bodies; and the behavior, under similar

conditions, of forms like the smuts, *Mucor*, and *Penicillium*, would make advisable a larger measure of caution in dealing with fungi growing irregularly in a submerged condition.

Although various details associated with the sporulation of *Actinomyces* have thus been dealt with in the literature, the opinion still seems to prevail widely that the process is of an irregular and miscellaneous nature. LITMAN and CUNNINGHAM (14) in recent years have denied the character of spores to the "gonidia" produced by the potato-scab organism; the elements are believed simply to "serve as a segment of the mycelium, which, by increasing the number of segments, may increase the chances for spread and continuous existence." This view seems, in the opinion of the writer, very much at variance with the distinctiveness of the well characterized sporogenous apparatus found in *Actinomyces*.

In pursuing the present studies a method of mounting material was employed which, in view of the exceptional fragility of all species of *Actinomyces*, and the great difficulty ordinarily encountered in attempting to stain undisturbed sporulating conditions, gave exceptionally good results. The plants were grown on a suitable substratum, usually potato or glucose agar. Growth on potato agar, as a general rule, is more prompt and productive of mycelium; but as its use, especially with species exerting a strong tyrosinase reaction, stimulates to excessive guttation and disruption of the sporophores by the extruded droplets, a medium not possessing this property is often found to be advantageous. After the cultures had attained a proper degree of maturity, the whole growth was cut from the agar and removed from the tube as carefully as possible. A slide smeared with albumen fixative was now brought into firm contact with the mycelium and then separated from it, precautions being taken to avoid altogether any sliding of the two surfaces on each other. If the growth is not too young, this procedure will leave the upper portions of the aërial mycelium adhering to the slide without serious disarrangement, and killing and fixation may be at once effected by the use, for example, of strong alcohol. The material was subsequently stained and mounted in balsam. The quality of preparations in which the spore chains have commenced to disintegrate in large numbers is

impaired by the presence of large masses of free spores, which retain their staining properties for some time after maturing. Later the spore walls seem to become entirely impervious to stains, and as a result when the secondary mycelium develops beyond a slight clouding effect no difficulty is encountered from this source. The best results are obtained when the print is made soon after the mycelium begins to adhere readily to the smeared slide.

The nature of the killing agent employed was found to have no noticeable effect on the preparation. Flemming's mixtures, both weaker and stronger, picro-formol, picro-acetic, Carnoy's fluid, and 95 per cent alcohol were tried with apparently the same results. Owing to the small diameter of the filaments, the penetration is probably so nearly instantaneous that plasmolysis is effectively prevented by nearly any toxic agent capable of readily wetting the material. In order to obviate the necessity of washing, strong alcohol was used almost exclusively.

Much more depends on the proper choice of a stain. Saffranin, gentian violet, Bismarck brown, and eosin usually fail to bring about a sufficiently deep coloration. Carbol-fuchsin acts powerfully and rapidly, but is poor for purposes of differentiation. Haidenhain's iron-alum haematoxylin is good for protoplasmic structures. The most satisfactory results were obtained with Delafield's haematoxylin, which if allowed to act for 24 hours, with the proper degree of decolorization, yields deeply stained, clear preparations, showing vacuoles, metachromatic, and nuclear structures, as well as septa, with remarkable distinctness.

The spores of all species of *Actinomyces* are developed by a transformation of more or less specialized hyphal branches distinguishable from the sterile hyphae of the aërial mycelium at an early stage in their development. In general, with the exception of such inflated hyphae as are shown in figs. 47, 48, and 106, the diameter of any portion of sterile mycelium is attained at the time it arises through the elongation of the growing filament tip, subsequent increase in thickness being very slight. The sporogenous branches, however, are in the beginning conspicuously thinner than the axial hyphae from which they are derived. Later, when their final linear extension has been nearly attained, increase in thickness

generally follows. This increase may be slight, as in some species in which the mature sporogenous hyphae are still somewhat thinner than the vegetative hyphae (fig. 46), or more considerable, as in forms in which they conspicuously exceed the latter in thickness (figs. 4–6). The very simple type represented by *Actinomyces* XIII, in which the aërial mycelium is represented by very long filaments, rarely branching and apparently sporogenous almost to their point of origin in the nutritive mycelium, constitutes the only exception, since in this instance there is no indication of thickening in the young fertile hyphae, nor indeed any variation in the diameter of its vegetative filaments.

In a majority of the species the maturation of the sporogenous hyphae is associated with a peculiarity in growth by which they become coiled in more or less characteristic spirals. The tendency toward the coiled condition is usually clearly manifested before the branch has grown to half its final length through the open flexuous habit of the young filament (figs. 5*c2*, 107). As elongation continues, the turns become increasingly definite, but the contraction leading to the final condition, which ranges from that illustrated by *Actinomyces* XIII with its open, barely perceptible turns, to one in which the spirals are so strongly compressed that its adjacent turns are in continuous contact (figs. 44, 51, 57) in a fashion resembling that of the spores of the hyphomycetous genus *Helicoön*, is usually delayed until the later growth in thickness of the filament. Specific differences may not only be indicated by the obliquity of the spiral, but involve also the number and diameter of its turns, and its construction with reference to the dextrorse or sinistrorse condition. The range in different species extends from the 2 or 3 turns exhibited in forms like *Actinomyces* II and XVI, to over 20 turns in others; but the range in a particular species is always considerably smaller. The writer once observed a spiral with 24 turns, but this probably approximates the extreme maximum; spirals with 14–16 turns (figs. 23, 57, 94*c*) are by no means abundant, and probably no species produces many in which there are more than 12 turns.

The diameter of the spirals as a whole is more or less in inverse ratio to the number of turns characteristic of the species. This

correlation is very evident in a comparison of types like *Actinomyces* II and XVI showing spiral sporogenous hyphae with a few wide turns, and types like IV, V, and XVII illustrating spirals of many narrow turns.

Rotation in the formation of spirals is specifically sinistrorse or dextrorse in different species of the genus; and it is interesting to note that here, as in the vegetable world in general, sinistrorse are much more abundant than dextrorse species. Of the 17 species with spiral sporogenous branches figured in the present paper, which have been selected as representative of a much larger number, 5 are dextrorse, 11 are sinistrorse, while the condition of the remaining one could not be ascertained with certainty. In general, the proportion appears throughout the entire genus. As a morphological feature, the absolute constancy with which a species adheres to one kind of rotation is noteworthy, particularly in view of the extremely minute dimensions of the structures concerned.

An examination and comparison of the relation of the sporogenous branches to each other, and to the axial filaments, enables one to recognize several tendencies, the distinguishing characteristics of which are correlated with differences in the sequence of proliferation. Two main types may thus be recognized, approaching each other in apparently intermediate forms, but moderately distinct at the extremes: (1) an erect dendroidic type in which the sequence of development of the sporogenous hyphae is successive; and (2) a prostrate, racemose type in which the development is more nearly simultaneous.

In the erect type, well exemplified in *Actinomyces* I, the development of the fructification starts from a single erect hypha with a spiral termination. Sporogenesis commences at the tip by the insertion of regularly spaced septa, and proceeds downward toward the base of the filament. Usually before much of the hypha has been involved, a single septum will appear well toward its base, and immediately below it the bud anlage of a new sporogenous branch appears. As the latter is attaining its growth in length and thickness, and its spiral disposition, the basipetal septation in the axial filament proceeds to the septum above the insertion of this first branch, the young spores thus delimited undergo maturation

processes, the spiral becomes relaxed, and the chain of spores subject to disruption. The branch now passes through the same course of development as the axial filament and in turn gives rise to a sporogenous branch below a septum a little above its own insertion. The number of sporogenous branches developed below a single septum is generally increased to several by proliferations subsequent to the first; and as the initiation and development of successive orders may be indefinitely repeated, complex fructifications are frequently developed, in which a succession of the processes described are simultaneously taking place at many points.

In the second type there is no such clearly defined relation between younger and more mature sporogenous hyphae. Development of a fructification is initiated by the proliferation of branches at irregular intervals on the distal portion of a prostrate axial filament which often exceeds 1 mm. in length. The branches may either stop their more extensive development after forming a spiral, or themselves proliferate a secondary branch a short distance above their own insertion; and this in turn may form a spiral and give rise to a tertiary branch (fig. 43). By a repetition of this process each lateral element may become branched several times, the whole apparatus as well as its insertion on the axial filament being characterized by an absence of septa. Sporulation, instead of beginning in any individual spiral as soon as it is formed, is usually delayed until the branching and growth of spiral hyphae in the same lateral process have come to an end (figs. 42–44, 46), when it will often proceed rapidly and almost simultaneously in all the spirals (fig. 41). The termination of the axial filament itself develops into a spiral, and behaves essentially like a primary lateral branch.

Occasionally the axis of one of these racemose arrangements may be comparatively short, resulting in a rather intricate structure in which the spirals of one lateral branch may be entangled with those of another (fig. 44). The tendencies characteristic of the type, however, are maintained: the absence of a septum above the insertion of branches, and the delay in sporulation in the spirals first formed, until the growth of the last order of sporogenous branches is more or less complete.

In addition, however, to species in which these two types are clearly distinguished, a still larger number of species present a combination of the two features. Frequently the open racemose arrangement of the lateral branches on the main axial filament is associated with a successive order of development in the further ramifications of the branches (figs. 63, 79). The presence of a septum above the insertion of a branch is characteristic of more species than is its absence (figs. 2, 5*c*); and in some species both conditions prevail (figs. 53, 79, 81). In other forms a fructification with successive development may terminate a long prostrate filament.

In a few species, particularly *Actinomyces* X and XVIII, there are formed, in addition to the more regular fructifications, others of a more miscellaneous tendency. The branching axial filaments are relatively thick, densely filled with protoplasm, and bear at very close and irregular intervals a short, thick, unbranched sporogenous hypha with little or no spiral modification (fig. 103). It seems quite probable that this type of development is associated with the excessively rapid growth that characterizes the two forms in which it was most frequently observed.

The degree of completeness to which the aërial mycelium of *Actinomyces* is converted into spores has generally been overestimated. On the contrary, sporulation is quite strictly confined to terminal elements, never as a rule passing beyond the first junction with another element. The proliferation of the branch nearest the end of the axial filament limits spore production in this filament to the portion beyond the insertion of the branch; and in the same manner the proliferation of a secondary from a primary lateral branch results in a sterilization of the portion of hypha below the insertion of the new branch. In one species, *Actinomyces* V, sporulation is even further restricted by the apparent abortion of a number of potential spores at the proximal end of the unbranched lateral branches. The hyphal portion involved first develops as usual, but when the characteristic septation associated with the delimitation of spores in this species appears in the spiral, it is not extended to the base of the branch, although indications of regularly spaced membranes may usually be distinguished (figs. 21,

24, 25). Later the unsegmented portion is gradually evacuated and converted into a sterile stalk devoid of protoplasm (figs. 29, 30). It is interesting to note that the basal septum, which in an allied and very similar form, *Actinomyces* VI, delimits the lowest spore from the axial filament, here also is present as a well developed cross-wall.

The delimitation of the ultimate cells in the process of sporulation occurs usually as the growth in thickness and the contraction of the spiral (where this is present) are approaching a stage of completion. It has usually been believed by investigators that the details connected with spore formation are uniform throughout the genus. This belief, which the writer was at first inclined to share, must be considerably modified in view of the diversity of conditions actually found. In most species the sporogenous hyphae become divided into regular cylindrical cells separated by septa; the latter generally stain deeply with Delafield's haematoxylin, probably as the result of an association with metachromatic or possibly nuclear material. The species which are thus characterized by clearly defined septation may be assigned to three different categories, separated by differences in the disposition of their septa and in the development of their spores.

In the first category, represented by *Actinomyces* I, the cross-walls in the sporogenous hyphae remain without any very pronounced change, continuing to separate the adjacent cells until these have developed into a chain of mature contiguous spores. The insertion of these septa progresses from the tip toward the base, and does not break the physiological continuity of the hyphae; for food material apparently is readily transported through them to the young spores at the termination, since these subsequently increase in size, and may deposit a wall of measurable thickness.

In the second category the septa apparently split into halves, which are then drawn apart by the longitudinal contraction of the individual protoplasts (figs. 5*c1*, 8*a–f*, 59). In *Actinomyces* II the very pronounced growth in thickness of the sporogenous hyphae, following the insertion of septa, indicates that in this species also septation brings about no impediment in the transfer of food material. This is particularly remarkable on account of the

extraordinary thickness of the septa characterizing this species. *Actinomyces* XVII, however, while less striking, probably represents more nearly the usual condition prevailing in the second category. The segment of the filament wall evacuated by the contraction of each two successive spores undergoes no change until fractured by the disruption of the chain of mature spores.

In the third category (*Actinomyces* IV, V, VI, VII, and XII) the cross-walls first undergo a deep constriction, which by involving the ends of the young cylindrical spores gives to the latter an elongated ellipsoidal shape (fig. 70*a–d*). The constricted septum now gradually loses its staining properties, and appears to become slightly drawn out in a longitudinal direction (fig. 70*e*). A preparation stained with Delafield's haematoxylin usually shows many old spore chains in which the individual spores are thus connected by hyaline isthmuses. Occasionally an isthmus may be found with a remnant of the old deep staining septum still unchanged in its center (figs. 16, 70*e*).

Beyond these three types of sporulation another must at least be provisionally recognized, in which septa are either absent from the developing sporogenous hyphae, or are not demonstrated by the use of ordinary stains. The protoplast appears to contract at regular intervals, yielding a series of non-contiguous spores, held together for a time by the connecting segments of evacuated filament wall (fig. 73). It is this type of sporulation which NEUKIRCH and his followers, in opposition to LACHNER-SANDOVAL, believed to prevail throughout the genus. NEUKIRCH'S conclusion that septa are never involved in the sporogenesis of *Actinomyces* certainly cannot be extended to the large majority of species; and its application to any forms whatsoever is associated with some reasonable doubt. The writer is inclined to believe that cross-walls appear in the development of the sporogenous hyphae probably throughout the genus, but in some members are too thin to be recognized as distinct septa. Such an interpretation is suggested by the wide range in the thickness of septa found to occur, from the very massive structures of *Actinomyces* II, through those of moderate thickness in *Actinomyces* I, XII, and XVI, to the condition prevailing in

Actinomyces III and VIII, where cross-walls can only rarely be distinctly perceived.

All investigators, with the exception of SCHÜTZE, agree in attributing to the peripheral wall of the filament of *Actinomyces* an extreme thinness. Indeed, KRUSE (11) and others have urged the single contoured character of the membrane as an evidence of the bacterial affinity of the genus. It is only necessary to examine fungus forms like *Chlorosplenium* or *Phoma*, to convince one's self that the single contoured wall is generally characteristic of minute cells, whatever their taxonomic connections may be. Yet while the phylogenetic inference may safely be rejected, it still remains true that the peripheral wall of every species of *Actinomyces*, except possibly those of some old enlarged hyphae, cannot be made out as a distinct structure with double contour. In evacuated portions its location is indicated by only the faintest indication of its outline. Nor is this surprising when we consider that the maximum resolving power of any combination of lenses employing visible light is approximately 0.17 μ. As this magnitude barely equals the widths of the thinnest cross-walls observed, it is not difficult to suppose that, in the type of sporogenous hyphae represented in *Actinomyces* XIII, the dimensions of the partitions, like the filament wall generally, fall below the limit of visibility.

It is pertinent in this connection to emphasize the peculiarity in the nature of the cross-walls, the appearance of which in many species of *Actinomyces* initiates the development of the individual spores. Their unusual relative thickness, even in species in which they can be distinguished only with difficulty, but where nevertheless their thickness must exceed 0.17 μ, in filaments with a diameter of only 0.9 μ, is indicative of a composition essentially different from that of the peripheral wall. This indication is strengthened by the strong affinity for dyes characteristic of the septa, the evident ease with which they permit of the passage of food material, and their apparent plasticity of behavior, resulting in a median split in some species, and in others in a gradual constriction followed by a slow transformation into an attenuated isthmus.

The disappearance of the deep staining derivatives of the septa from the ends of the young spores is in some species accompanied

by the appearance of one or several deep staining granules within the spore. Whether the latter represent nuclei or bodies of metachromatic material cannot definitely be determined. It seems not at all improbable, however, that some of the structures that can be differentiated within the more mature spores, particularly those characterized by uniform size and moderate staining properties (figs. 1, 2, 33, 35) are nuclei. In *Actinomyces* IV and XII they may frequently be distinguished comparatively early, before the septa, with which they alternate in regular succession, show any perceptible constriction, indicating that their existence is not related to the subsequent disposition of the partitions (fig. 67). When two of these bodies occur in the same spore they uniformly occupy opposite or diagonal positions (fig. 2, *a*, *d*2). The question arises why these bodies, if they actually represent nuclei and not structures originating *de novo*, cannot be distinguished in the young continuous sporogenous hyphae. The only explanation that can be advanced is that the protoplasm in the earlier stages is too dense to make possible any conspicuous contact between cytoplasm and nucleus. Later, with the attenuation and vacuolization of the cytoplasm that occur with the maturation of the spore, apparently as the result of the deposition of a special wall, the nucleus becomes increasingly distinct, and in some species it constitutes the only spore structure clearly visible in the stained preparation (fig. 41).

It cannot be denied, however, that granules having more the appearance of the metachromatic granules found in degenerate sterile filaments occur in the spores of some species, either alone or together with a nucleus-like body. They differ from the latter in taking a deeper stain; in having an absolutely smooth contour; in offering considerable variability in size; and when present in numbers assuming no definite orientation with reference to each other. They have been noted in those species in which the septa associated with the delimitation of spores is particularly massive; and in *Actinomyces* II (fig. 8*f*) their derivation from the excessive wall material seems reasonably well established. After the septa have separated along a median plane, the deep staining substance at each end may contract, yielding a number of spherical bodies inside of the spore. This process is probably of a more or less

pathological nature, since in the usual type of development the wall material is gradually distributed through the inclosed protoplasm, causing the normal mature spore, except for the presence of a vacuole, to take an almost homogeneous stain.

Another indication of the similarity in nature existing between metachromatic material and the deep staining transverse septa of *Actinomyces* is found in the occurrence of both within peculiar large spherical structures. These structures appear generally to occupy nearly the entire lumen of the filament, and not infrequently are related to local enlargements. Occasionally, however, their diameter is considerably smaller than that of the hyphae (fig. 103). In any case they may contain either one or several peripherally located metachromatic granules, or a uniformly thick, well defined, deep staining, transverse septum, exactly median in position. It is interesting to note that whenever granules occur their surfaces in contact with the periphery of the structure represent portions of convex spherical surfaces conforming accurately to the confining surface; and whenever a septum is found traversing one of these structures considerably smaller in diameter than the filament, it does not extend into the protoplasm, but remains in its finished state as a curious partial partition.

The germination of the spores of *Actinomyces* takes place readily in dilute nutrient solutions, such as 1 per cent glucose solution, or nearly any vegetable decoction. During the first few hours of incubation at a moderate temperature they increase considerably in volume by swelling. From 1 to 4 germ tubes are then produced, apparently more or less successfully, the approximate number being, in a measure, characteristic of the species. Specific characteristics are expressed also in the diameter of the hyphae, and in the frequency of branching.

Cryptogamic Laboratories
Harvard University
Cambridge, Mass.

PROTOMARATTIA, A NEW GENUS OF MARATTIACEAE, AND ARCHANGIOPTERIS

(WITH PLATE I AND THREE FIGURES)

BUNZO HAYATA

The Marattiaceae constitute a small family which may be regarded as the survivor of a much larger group. At present only 6 genera are known: *Archangiopteris*,[1] *Macroglossum*,[2] *Angiopteris*, *Marattia*, *Christensenia* (*Kaulfussia*), and *Danaea*. *Archangiopteris* was first discovered by A. HENRY in Yunnan and was published in 1899 by CHRIST and GIESENHAGEN as a genus connecting *Angiopteris* with *Danaea*. The type of the genus *A. Henryi* is shown to be one of the most primitive forms of Marattiaceae by its simply pinnate leaves and simple stelar structure.[3] According to GWYNNE-VAUGHAN,[4] the mature stem of *Archangiopteris* retains a stage which is rapidly passed through by the young plants of *Angiopteris* and other genera. The same seems to hold true as to the form of the leaves. As far as I have observed *Angiopteris* in its habitat in Formosa, the first 2 or 3 leaves from a young stock usually are simply pinnate, but die before they reach maturity and become fertile. The pinnae of these first leaves are much larger than of those that follow, and closely resemble those of *Archangiopteris* in shape and size. We may infer, therefore, that *Archangiopteris* represents the form of a primitive type to which the ancestor of *Angiopteris* may have belonged.

Archangiopteris is most closely related to *Macroglossum*,[5] recently established by COPELAND, both in the simply pinnate fronds and

[1] CHRIST, H., and GIESENHAGEN, K., Pteridographische Notizen. Flora 86:72–85. 1899.

[2] COPELAND, E. B., The ferns of the Malay-Asiatic region. Philipp. Jour. Sci. 4:1–64. 1909.

[3] LOTSY, J. P., Vorträge über botanische Stammesgeschichte 2:676. 1906.

[4] GWYNNE-VAUGHAN, D. T., On the anatomy of *Archangiopteris Henryi* and other Marattiaceae. Ann. Botany 19:268. 1905.

[5] CAMPBELL, D. H., The genus *Macroglossum* Copeland. Philipp. Jour. Sci. 9:199–223. 1914; The structure and affinities of *Macroglossum Alidae* Copeland. Ann. Botany 28:651–669. 1914.

in the elongated linear sori, but differs from it in the absence of ridges between the sori and in the dorsiventral rhizomes. GWYNNE-VAUGHAN (*loc. cit.*) says that in the specimens examined by him there was no suggestion of dorsiventrality in the rhizome of *Archangiopteris*, and the leaf arrangement and the vascular structure indicated a radial symmetry. So far as I can judge from the figure given by the author,[6] however, there are some indications of dorsiventrality in the rhizome, as can be seen in the upward bending of the stipes of the leaves and in the fact that the rhizome ascends somewhat obliquely toward the apex; and it also may be inferred that the remaining portions of the rhizome not given in the figure very probably run horizontally. In *Archangiopteris Somai*, recently discovered in Formosa, and two other new species from Tonkin, which will be described later, the rhizomes are prostrate and show very clear signs of dorsiventrality.

In the summer of 1916 I was sent to Tonkin for collecting. I found there two new species of *Archangiopteris* and a type of a new genus closely related to the latter. All these plants present an appearance very similar to other ferns, such as *Coniogramme japonica*, *C. fraxinea*, *Diplaziopsis javanica*, or *Diplazium bantamense*, and occur in very small numbers amidst a multitude of the previously mentioned ferns. As it is very rarely that one has the opportunity to meet with these plants of *Archangiopteris* and the allied new genus, it may not be entirely out of place if I should tell how I was led to discover these very rare and interesting ferns.

Some 10 years ago I was greatly interested in learning of HENRY'S discovery of *Archangiopteris*, representing as it did an ancient type of the Marattiaceae, and I wondered whether there might not exist another species of the genus in Formosa, the flora of which I have since then been studying. In 1915, when examining collections sent by the late T. SOMA from Formosa, I found among them a curious looking fern labeled *Gymnogramme japonica*. A glance at the specimen showed me that it was another type of *Archangiopteris*, which was then named and published as *A. Somai*.[7]

[6] GWYNNE-VAUGHAN, D. T., *loc. cit. pl. 10. figs. 1* and *2*.

[7] HAYATA, B., Icones Plantarum Formosanarum. 5:256; 6:154. *pl. 19.*

The next year I went to Formosa to the native place of the plant on the bank of a rivulet in a dense forest at an altitude of about 2000 ft. in the northern part of the island, for I wished to see a living specimen of this highly interesting fern. On the first day of our search we were not successful. The difficulty of finding it is partly due to its extreme rarity and partly to its existence only among other ferns closely resembling it in external appearance. On the second day I was at last successful in finding a few specimens of *Archangiopteris Somai.*

In the summer of 1917 I went to Chapa in the mountainous regions on the boundary between Yunnan and Tonkin. There too I wondered if I might not have the opportunity of finding some *Archangiopteris*, and so I made a careful search, turning back the leaves of all similar ferns which I came across. At last, as I had expected, I found a stock of the desired genus, in the shade of the forest at an altitude of about 4000 ft., between Chapa and Mueng-Xen. It was just a single stock. The fern resembled *A. Henryi*, but differed from it in the absence of indusium scales in the middle of the sori. It was a new species which I propose to call *A. subintegra*, a description of it being given in the present paper. Later on I went to Mt. Tamdao in the central part of Tonkin, and collected in a forest at an altitude of about 3000 ft. There I saw on the side of the forestry service path one poor specimen which I thought most certainly a species of *Archangiopteris* before examining the fern. It was a sterile specimen, yet I believed it to be a plant of the same genus, until I found near by some fertile specimens. They revealed the fact that they were different from *Archangiopteris* in fructification, all other characters being exactly like the latter. The sporangia of the newly discovered plant were quite fused together, reminding one exactly of those of *Marattia*, but differing from the latter in the long linear synangium. I thought that it might be a type of a new genus intermediate between *Archangiopteris* and *Marattia;* but I shall refer to this later. Not very far from there I collected a true *Archangiopteris*, another new species, which I propose to call *A. tamdaoensis. Archangiopteris*, therefore, formerly a monotypic genus, has come to comprise 4 species. As to distribution, the species are extremely local.

A. Henryi is only known from Mentzu (Yunnan); *A. Somai* exists in one or two spots in the northern part of Formosa; *A. subintegra* occurs in one place in the mountains of Chapa between Yunnan and Tonkin; and *A. tamdaoensis* is found in one locality on Mt. Tamdao (Tonkin); and the new genus is also limited to one spot on Mt. Tamdao.

Returning to the systematic position of the new genus, the most remarkable feature which separates the new type from all the other genera of the Marattiaceae is its elongated linear or even vermiform synangium. The other important characters are its horizontal dorsiventral rhizomes and simply pinnate fronds. Through the dorsiventral rhizomes it is related to *Kaulfussia* and *Archangiopteris;* by the simple pinnate fronds it is allied to *Macroglossum*, *Danaea*, and *Archangiopteris;* in the structure of the synangium its nearest of kin is to be found in *Marattia*. It is distinguishable from the latter, however, by its elongated linear synangium, simply pinnate fronds, and dorsiventral horizontal rhizome. It differs from *Archangiopteris* in having a synangium; from *Macroglossum* in the dorsiventral rhizomes and synangium; from *Angiopteris* in the simple pinnate leaves, dorsiventral rhizomes, and synangium; from *Danaea* in the synangium with a longitudinal common slit; and from *Kaulfussia* in the pinnate leaves and linear synangium. After considering all these cases, I am forced to the conclusion that the new plant must be a type representing a new genus. I propose to call it *Protomarattia*, as it bears exactly the same relation to *Marattia* as *Archangiopteris* does to *Angiopteris*.

As was stated, *Protomarattia* closely resembles *Marattia* in the reproductive organs, while it is closely related to *Archangiopteris* in its vegetative organs. The similarity of the type of the new genus and *Archangiopteris tamdaoensis* in the fronds and rhizomes, even in the serration and venation, is really so very great that I entirely failed to distinguish the one from the other until I saw the sori. The protective arrangement of stipules and commissures of our plant is exactly like that in *Archangiopteris*. The synangium also, presenting a linear form, with comparatively thinner lateral walls and a little looser connection of locules, more or less tends toward the sorus of *Archangiopteris*, or even toward that of

Macroglossum.[8] There can be no doubt, therefore, that the genus is closely related to *Marattia* on the one hand, while on the other it is nearly allied to *Archangiopteris*. It represents presumably a form of an ancient and conserved type connecting *Marattia* with *Archangiopteris*.

Protomarattia, nov. gen.—Rhizoma dorsiventrale oblique vel horizontaliter prostratum, reliquiis stipitum dense obtectum, radicibus e latere ventrali oriundis. Folia circum rhizoma spiraliter disposita; stipulis e latere adaxiali stipitis commissura connexis; stipitibus rectis duobus locis basi et superiore geniculato-incrassatis post finitas functiones e geniculo superiore et iterum basilari solutis; frondibus simpliciter pari- vel impari-pinnatis, pinnis patentibus vel interdum retrorsum reflexis; petiolulis pinnarum incrassatis. Synangium lineare submarginale e margine pinnae 4–6 mm. distans subsessile rima mediana longitudinali apertum; indusio e squamis numerosissimis laceratis inprimis constituto, demum evanescenti.

Differt a *Marattia* synangio lineari nec ovali, loculis multo numerosioribus, fronde multo minore simplicter nec pluries pinnata et rhizomate dorsiventrali repente nec erecto; differt a *Archangiopteris* synangio.

In Tonkin incola ad huc monotypica.

Protomarattia tonkinensis, sp. nov.—Rhizoma incrassatum horizontaliter vel oblique repens dorsiventrale, in specimine exsiccato nostro 15 cm. longum cum reliquiis foliorum 2.5 cm. latum, reliquiis stipitum et stipulis dense obtectum e latere ventrali radices incrassatas teretes emittens. Folia versus apicem rhizomatis 5–6 approximatim disposita. Stipites 30–40 cm. longi erecti planoconvexi in sectione duobus locis ad basin et ad 0.3 altitudinem a basi geniculato-incrassati, basi dense sursum pauce squamulati, squamulis lanceolatis vel linearibus circ. 2 mm. longis margine erosis; partibus incrassatis basilaribus geniculiformibus 2.5 cm. longis, 1.5 cm. latis utroque latere stipula et latere adaxiali commissura instructis, stipulis coriaceis tenuiter 2-lobatis, lobis abaxialibus (anterioribus) minoribus late semirotundatis, 1 cm. latis, 7 mm. longis margine erosis et membranaceis folium proprium (in

[8] CAMPBELL, D. H., The structure and affinities of *Macroglossum Alidae* Copeland. Ann. Botany 28:664. 1914.

alabastro) obtegentibus, lobis adaxialibus (posterioribus) majoribus semi-obovatis 1.5 cm. longis 7 mm. latis margine erosis et tenuibus cum commissura alabastrum folii juxta venientis obtegentibus; partibus incrassatis superioribus geniculiformibus, 2 cm. longis, 7–10 mm. in diametro sectionis in vivo nitidis; foliis post finitas functiones ex apice partis incrassatae basilaris et e parte incrassata superiore texturae degenerationi solutis. Frondes simpliciter pari- vel impari-pinnatae in ambitu obovatae vel ovatae stipitem in longitudine fere aequantes, circ. 30 cm. longae, 20–25 cm. latae, pinnis 4–5, rarius 3–7, alternis inferioribus minoribus superioribus majoribus, patentissimis vel interdum retrorsum reflexis, pinnis superioribus lanceolatis plus minus falcatis, 25–28 cm. longis, 5–6 cm. latis apice ad caudam abrupte acuminatis (cauda apicali lineari, 2–3 cm. longa, medio 4 mm. lata, serrata), basi subito triangulari-cuneatis (parte cuneata 1.5 cm. longa latere recta integra), margine planis nec recurvis nec undulatis minute serrulatis (serrula triangulari-acuta ascendenti), supra atroviridibus nitidis subtus pallidissimis subglabris ad costam et venas paucissime squamulatis (squamula minutissima), costa utraque facie elevata 1–2 mm. lata in exsiccato nigricanti; venis parallelis a costa angulo 80° egressis simplicibus vel e basi furcatis fere rectis prope marginem subite recurvo-ascendentibus a se 1.5 mm. distantibus ad apicem serrularum attingentibus apice haud incrassatis in exsiccato nigricantibus; petiolulis pinnarum circ. 1 cm. longis incrassatis squamulatis; rhachis frondis supra medio tenuiter sulcata in vivo angustissime alata 4–7 cm. longa; textura chartacea vel chartaceo-membranacea. Synangia numerosissima subsessilia secus venas vel venulas utroque latere costae prope marginem 1-seriatim approximatimque disposita, e margine 3–5 mm. ditantia, linearia vel vermiformia 4–6 mm. longa, 1 mm. lata rima longitudinali mediana aperta, primum squamulis laceratis indusiorum obtecta demum nuda, loculis numerosissimis utroque latere receptaculi 20–60 dispositis.

HABITAT.—Monte Tamdao (Tonkin), in silva ad 3000 ped. alt., leg. *B. Hayata*, July 1917.

ARCHANGIOPTERIS Christ et Giesenhagen, Pteridographische Notizen, Flora 86:72. 1899; BITTER, Natürliche Pflanzenfam.

1⁴:439; CHRISTENSEN, Ind. Filic. 62; CAMPBELL, Eusporangiatae, Publication no. 140, Carnegie Institution of Washington 203; LOTSY, Vorträg Bot. Stammesgeschichte 2:675.

KEY TO SPECIES

Indusium scales present between two rows of sporangia in a sorus....*A. Henryi*
Indusium scales absent between two rows of sporangia in a sorus.
Pinnae nearly entire, scales brown.....*A. subintegra*
Pinnae serrulate.
Scales dark brown or blackish.....*A. tamdaoensis*
Scales brown.....*A. Somai*

ARCHANGIOPTERIS HENRYI Christ et Giesenhagen, Pteridographische Notizen, Flora 86:77. 1899; GWYNNE-VAUGHAN, On the anatomy of *Archangiopteris Henryi*, etc., Ann. Botany 19:257–271. 1905.

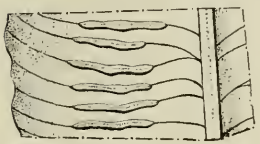


FIG. 1.—*Archangiopteris subintegra* Hayata.

HABITAT.—Mengtzu (Yunnan) ex HENRY.

Archangiopteris subintegra Hayata, sp. nov. (fig. 1).—Rhizoma horizontaliter vel plus minus oblique repens, in specimine nostro exsiccato 9 cm. longum cum reliquiis stipitum 4 cm. latum dorsiventrale reliquiis stipitum et stipulis persistentibus dense obtectum, radicibus incrassatis e latere ventrali rhizomatis oriundis. Stipes 70 cm. longus erectus basi dense sursum pauce squamulatus, squamulis lanceolatis, duobus locis basi et medio incrassatus, parte basilari incrassata 1 cm. longa totiusque lata stipulata, commissuris ut videntur obsoletis. Frons simpliciter pari- vel impari-pinnata in ambitu obovata, pinnis 5–7, superioribus majoribus lanceolatis, circ. 25 cm. longis, 5 cm. latis apice acuminatis ad caudas lineares abeuntibus (cauda 2–3 cm. longa serrulata), basi acutis margine cauda serrulata excepta fere integris; costa utraque pagine elevata, venis simplicibus vel a basi furcatis, venis et venulis parallelis a se 2.5 mm. distantibus patentissimis a costa angulo 85° egressis subrectis sursum plus minus recurvis; pagine supra nitida atroviridi, subtus pallidissima; petiolulis incrassatis 7 mm. longis squamulatis; textura membranacea. Sori lineares, 1 cm. longi, 1 mm. lati utroque latere costae 1-seriatim inter costam et marginem

dispositi a se 2.5 mm. distantes, primum squamulis filiformibus indusii obtecti demum nudi.

HABITAT.—Inter Chapa et Mueng-Xen (Tonkin), in silva ad 4000 ped. alt., leg. *B. Hayata*, July 1917.

Very distinct from the other members of the genus by the much thinner subentire pinnae.

Archangiopteris tamdaoensis Hayata, sp. nov. (fig. 2).—Rhizoma horizontaliter vel plus minus oblique repens dorsiventrale in specimine exsiccato nostro, 9 cm. longum cum reliquiis stipitum 3 cm. crassum e latere ventrali radices incrassatas teretes emittens, reliquiis stipitum et stipulis persistentibus dense obtectum. Stipes 40–45 cm. longus basi dense sursum pauce squamulatus, squamulis lanceolatis apice acuminatis vel ad acumen filiforme abeuntibus, 3–5 mm. longis, locis duobus ad basin et ad 0.3 altitudinem a basi incrassatus; parte incrassata basilari geniculiformi, 1–2 cm. longa, 1 cm. lata utroque latere stipula persistenti instructa, stipulis coriaceis latere adaxiali stipitis a commissura 2-fida connexis 2-lobatis, lobis anterioribus minoribus rotundatis 7 mm. latis totiusque longis margine erosis interiore recurvis, lobis posterioribus majoribus semi-oblongis, 2 cm. longis, 7 mm. latis margine erosis membranaceis; parte incrassata superiore stipitis geniculiformi, 2 cm. longa, 1 cm. lata; stipites post finitas functiones ex apice partis basilaris incrassatae et iterum e parte incrassata superiore texturae degenerationi soluti. Frons simpliciter pinnata in ambitu obovata pari- vel impari-pinnata, pinnis 3–4, superioribus majoribus lanceolatis, 23 cm. longis, 5.5 cm. latis apice gradatim acuminatis rarius subito acuminatis (acumine lineari 2.5 cm. longo, 2 mm. lato margine subintegro rarius serrulato), basi triangulari-acutis vel cuneatis margine praeter basin acumenque minute serrulatis (serrula triangulari-subacuta); costa utraque elevata, venis lateralibus numerosissimis parallelis patentibus a costa angulo 70° egressis subrectis simplicibus vel e basi furcatis, venulis ad apicem serrulatum attengentibus, a se 1.5 mm. distantibus; pagina supra atroviridi subnitida subtus pallidissima, supra glabra

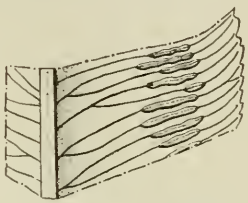


FIG. 2.—*Archangiopteris tamdaoensis* Hayata.

subtus paucissime squamulata vel glabra; petiolulis 5 mm. longis incrassatis. Sorus linearis utroque latere costae 1-seriatim secus venas vel venulas dispositus, 7–8 mm. longus, 1 mm. latus e costa circ. 1 cm. e margine circ. 4 mm. distans, primum squamulis filiformibus indusii obtectus demum nudus.

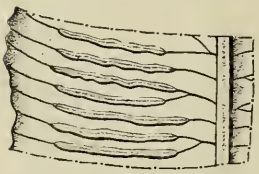


FIG. 3.—*Archangiopteris Somai* Hayata.

HABITAT.—Mt. Tamdao (Tonkin), in silva ad 3000 ped. alt., leg. *B. Hayata*, August 1917.

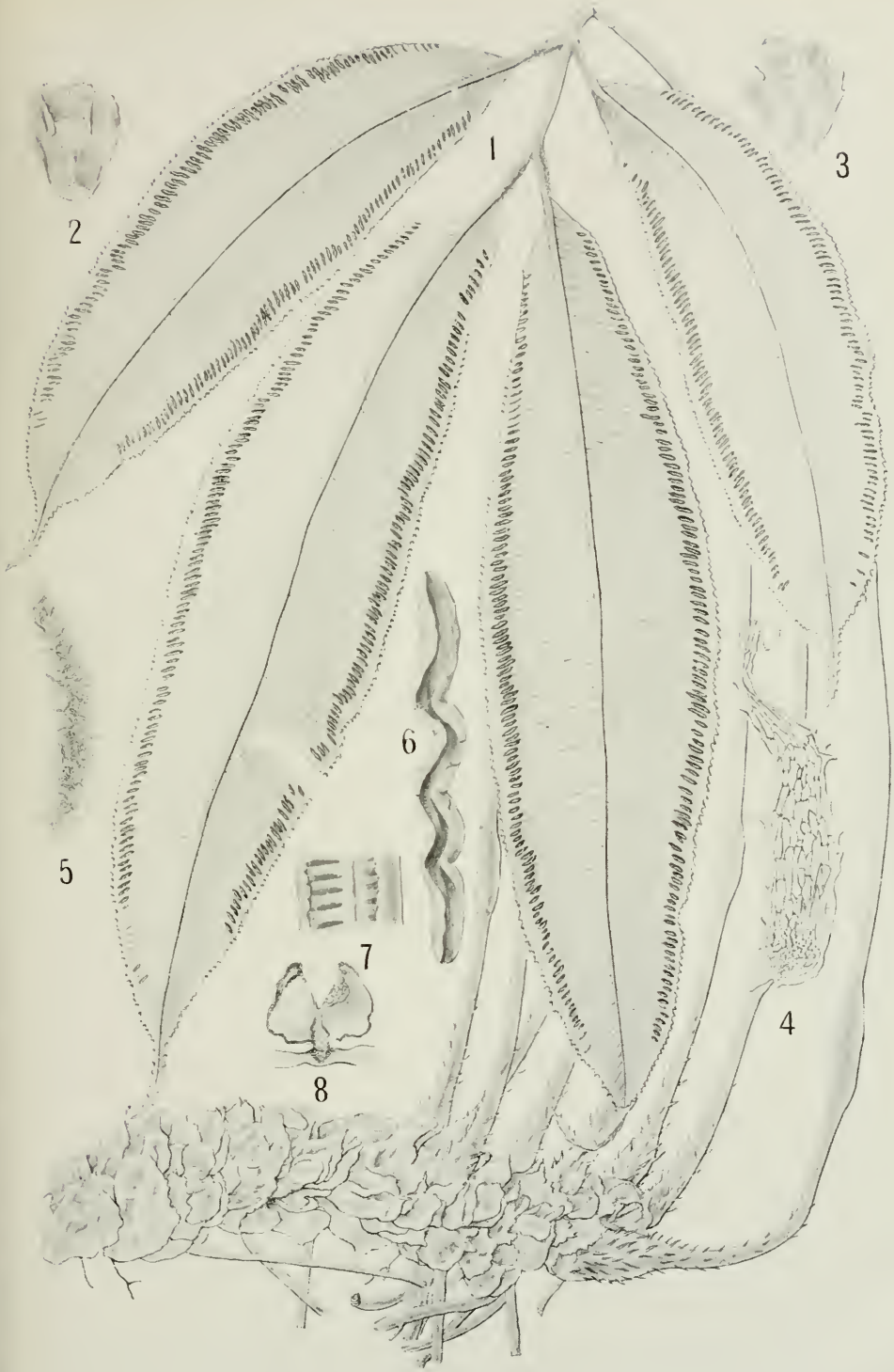
Allied to *A. Somai* Hay., but distinguishable from it in the shorter sorus located much nearer to the margin than to the costa, and in the less patent veins.

ARCHANGIOPTERIS SOMAI Hayata, Ic. Pl. Formosa 5:256; 6:154. *pl. 19* (fig. 3).

HABITAT.—Urai (Formosa).

EXPLANATION OF PLATE I

Protomarattia tonkinensis Hayata: *1*, plant; *2*, basal portion of stipe with stipules and a commissura, seen from abaxial side; *3*, same portion seen from adaxial side; *4*, scale on stipe; *5*, young synangium; *6*, full grown synangium; *7*, portion of same synangium showing chambers; *8*, section of same.



HAYATA on PROTOMARATTIA

CURRENT LITERATURE

BOOK REVIEWS

Fossil plants

The third volume of SEWARD's *Fossil plants*[1] will be welcomed alike by students of paleobotany and by those whose primary interest is in the morphology and phylogeny of the living vascular plants. The volume, comprising chapters xxvii–xxxix of the complete work, is devoted to Gymnosperms, the space being distributed as follows: Cycadales (recent) 34 pages, Pteridospermae 140, Cycadofilices 39, Cordaitales 86, Paleozoic gymnospermous seeds 66, Cycadophyta (fossil) 226, Bibliography of Vols. III and IV 48, Index 17, making a total of 656 pages. There are 252 figures, many of which are original.

The account of the living cycads, from the standpoint of a competent paleontologist, is particularly interesting and suggestive to one who, like the reviewer, is somewhat familiar with those forms, but is dependent upon investigators like SEWARD for descriptions of their extinct predecessors. This introductory chapter is a fitting introduction to the more detailed treatment of paleozoic and mesozoic members of the phylum. The practical advantage of such an introduction is sufficient excuse for treating the living cycads first instead of considering them in their natural place at the end of their phylum. The author believes the antiquity of that part of the cycadophyte phylum represented by the living cycads cannot be determined, but it is probable that if cycads, apart from Bennettitales, existed in the Jurassic and lower Cretaceous beds, they occupied a very subordinate place in comparison with the Bennettitales. While the living cycads resemble the Bennettitales in many vegetative features, we believe that the reproductive structures show a kind of difference which would make it impossible to derive the living cycads from any forms of the Cycadeoidea type; while, on the other hand, the Cycadofilicales, which SEWARD prefers to call Pteridospermae, have reproductive structures from which the cones of living cycads might easily be derived. If the living cycads have come from Bennettitales, they must have come from ancient types in which the megasporophylls still retained a distinct leaflike character. Whether they have come from the Bennettitales or directly from the Cycadofilicales, they must have greater antiquity than is indicated by any material yet discovered. We agree with SEWARD that the affinities are still in doubt, but we hope that Triassic material which can be sectioned will be found and that it will clear up relationships, for, it seems to us, the differentiation must have taken place long ago.

[1] SEWARD, A. C., Fossil plants, a textbook for students of botany and geology. Vol. III. Pteridospermae, Cycadofilices, Cordaitales, Cycadophyta. 8vo. pp. xviii+656. *figs. 253.* Cambridge University Press. 1917.

We should have treated the Pteridospermae, Cycadophyta, and Cycadales together as a cycadophyte phylum. The Cycadofilices, including fernlike plants which may belong to the Pteridospermae but in which seeds have not yet been discovered, naturally follow the known Pteridospermae; but it does not seem natural to treat the Cordaitales between the Pteridospermae and the Cycadophyta. After a careful reading of the Pteridospermae, we still fail to see why they should not be regarded as an order of the gymnosperms rather than as a group of equal rank. However, these are minor and very insignificant objections. The book is full of detailed descriptions and critical discussions which will make it possible for investigators with far less training than SEWARD to make valuable studies of such material as may fall into their hands.

The Pteridospermae are introduced by an excellent description of *Lyginopteris*, the name applied to the plant whose various fragments have been described under the names *Lyginodendron* (stem), *Sphenopteris* (leaf), *Lagenostoma* (seed), *Crossotheca* (microsporophylls), and *Kaloxylon* (root). The descriptions of *Heterangium* and *Medullosa*, while less complete, give a critical account of what is known up to date. The presentation of these 3 forms, with comparatively fragmentary accounts of others, shows where research is needed, and will enable students to fill in missing phases of life histories as material becomes available. In all the paleozoic forms of the cycadophyte phylum, information in regard to to the gametophytes and embryo, although very desirable, is very scant; but if attached seeds could be found and sectioned, the preservation seems good enough to show the desired features.

The treatment of the Bennettitales (Cycadophyta), although it occupies 226 pages, seems short in comparison with the big volumes of WIELAND. The English and French contributions to our knowledge of this group are presented in considerable detail, and the author has drawn upon WIELAND for numerous excellent figures. If well-preserved reproductive structures of the lower members of this group, especially *Williamsonia*, could be found and sectioned, the results could not fail to be important, for they would almost certainly throw light upon the origin of the living cycads.

The Cordaitales, representing the coniferophyte phylum, do not occupy so much space, but comparatively little is known about the group. If our knowledge of these forms were as complete as in case of the Bennettitales, a treatment of the Coniferales would be much simplified. As it is, the various stems, leaves, and reproductive organs referred to this group are described under their respective categories, and material is thus accumulating for a connected life history.

The chapter on paleozoic gymnospermous seeds is particularly conservative and interesting. Many morphologists would have felt little hesitation in assigning most of these seeds to one group or to another, but SEWARD, throughout the work, recognizes the danger of being too positive when dealing with unattached fragments. The characters of the various types of seeds are described and discussed. Although some knowledge of the internal structure is available, it is very evident that little is known in regard to the gametophyte. A knowledge of

the internal structure of the seeds, especially the smaller seeds, might help to connect the Cordaitales with the Pteridophytes.

The fact that the geographical distribution of plants at different stages in the development of the earth receives only disconnected treatment is excused by the plea that the space needed for Vols. III and IV (now in press) was underestimated, the original plan providing for a treatment of geographical distribution at the end of Vol. IV. However, SEWARD promises an entire volume devoted to this subject. Such a work would be welcomed by all students of morphology and phylogeny, and we hope that the volume will make its appearance at an early date.

The complete bibliography and index, together with the critical and conservative presentation of the entire subject, make the work indispensable to those engaged in research upon fossil plants.—C. J. CHAMBERLAIN.

NOTES FOR STUDENTS

Chlorophyll inheritance.—This subject seems to be a stumbling-block both for plant geneticists and cytologists. In 1913 EMERSON and EAST[2] stated that there were on record only two indisputable cases of non-Mendelian inheritance. Both of these were cases of chlorophyll inheritance. CORRENS[3] made reciprocal crosses of a variegated *Mirabilis* (*albomaculata*) with normal green plants, and discovered that in this case inheritance was strictly maternal, the pollen evidently contributing nothing. He explained this by assuming that the variegation was due to a disease of the cytoplasm which destroyed many of the chloroplasts, and that nuclei were immune to this disease. Thus the disease could be transmitted to progeny by the female parent only, since the male is supposed by cytologists to contribute only a nucleus stripped free from its cytoplasm. If one grants CORRENS' assumptions, the mechanism provided will explain this case of maternal inheritance without any violation of MENDEL'S law, for here there would be no true inheritance, but merely reinfection.

BAUR,[4] working with a *Pelargonium* which had white-margined leaves, observed an occasional pure green branch and an occasional pure white branch. Flowers on these branches when self-fertilized gave respectively pure green and pure white progeny (the latter, of course, dying in the seedling stage). A cross either way between the two branches resulted in progeny which were a mosaic of green and white. Such behavior can be accounted for by either of two explanations, but each involves a very bold assumption. If there is a Mendelian determiner responsible for the full green development, and a white

[2] EMERSON, R. A., and EAST, E. M., Inheritance of quantitative characters in maize. Bull. Agric. Exper. Sta. Nebr. no. 2. pp. 120. *figs. 21.* 1913.

[3] CORRENS, C. E., Zeitschr. Ind. Abstamm. Vererb. 2:331–340. 1909.

[4] BAUR, ERWIN, Zeitschr. Ind. Abstamm. Vererb. 1:330. 1909.

plant lacks that determiner, it would not be an unheard-of thing for a cross between the two to show a mosaic (particulate inheritance). But for pure green and pure white branches to form and breed true sexually would involve somatic segregation. Such an explanation is hard to accept, since we have been confident not only that no general reduction division ever takes place in somatic tissue, but also that segregation in individual pairs of chromosomes or parts of chromosomes is impossible elsewhere than at spore formation. We might accept such a possibility for very rare monstrosities, but the case in hand seems to be a matter of fairly regular behavior. Mutation might also account for these results, but this too could hardly be expected to take place with such regularity.

The other explanation seemed much more reasonable to BAUR, but that too he acknowledged to be unorthodox. He assumed that this was not a matter of chromosomes but of plastids, and of course somatic segregation of green and white plastids is quite reasonable. If this mechanism be the true one, however, one must also grant that plastid initials are contributed by the male parent. This last is quite unorthodox and seems flatly contradictory of CORRENS' ideas, for if enough cytoplasm is contributed by the male to introduce plastid initials, why should it not also contribute the diseased condition of CORRENS' *albomaculata?*

IKENO,[5] working on variegated races of *Capsicum*, confirms BAUR'S qualitative results, and makes the case still stronger by uncovering some very significant quantitative features. "The offspring arising from the hybridization between a variegated and a green plant in either of two reciprocal ways contain a relatively far larger number of slightly variegated (less white) plants than those arising from the self-fertilization of the same variegated plant." The intensity of variegation may be progressively diminished by repeated crosses with green plants, but not even a single self-colored green has as yet been obtained in that way. IKENO concludes that the transmission of variegation is not through the nucleus, but through the plastids in the cytoplasm; the male contributes cytoplasm and plastids.

It seems impossible to reconcile this behavior with CORRENS' maternal inheritance. To assume that plastid initials originate within the nucleus might smooth over the immediate difficulty, and would carry us into further complications. A more hopeful suggestion is that the disease which CORRENS speaks of attacks only mature chloroplasts and that plastid initials are immune, as well as the cytoplasm around them. The easiest assumption, of course, would be to claim that CORRENS overlooked a case of apogamy. Otherwise we may be driven to acknowledge that chlorophyll inheritance in angiosperms is governed by at least two mechanisms, which are not only quite different but directly contradictory.—MERLE C. COULTER.

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[5] IKENO, S., Studies on the hybrids of *Capsicum annuum*. II. On some variegated races. Jour. Genetics 6:201–229. *pl. 8. figs. 1, 2.* 1917.

Gonidia of lichens.—In 1905 ELFVING, of the University of Helsingsfors, published his studies, which he thought disproved the recent view that the chlorophyllous elements associated with lichens are algae. He continued his work and published his results in 1913. In the interval DANILOV[6] began studies which disproved ELFVING's conclusions. DANILOV's results were published in Russian in 1910 and in English in 1918.[7] ELFVING's conclusion was that the lichen hyphae threw out spherical cells, at first colorless, but later colored and very similar to algae. These he supposed became separated from the hyphae and divided rapidly within the lichen thallus, forming, according to his results, the "gonidia" of lichens. Reviewing these results, DANILOV found on careful study that unstained preparations often left the impression that the algal cells might really be outgrowths of the lichen hyphae, with which they are intimately associated. By the use of stains, however, he was able to trace the entrance of the hyphae into the algal cells, thus proving that there is no genetic relationship, but that the relationship is rather that of host and parasite. The "pale gonidia" of ELFVING were found to be dead algae which had been killed by the parasitic lichen, and DANILOV was able to see distinctly the lichen hyphae within them.

Important and quite apart from the refutation of the once generally accepted view of the origin of the chlorophyllous "gonidia" from the non-chlorophyllous lichens, are the conclusions of DANILOV regarding the relation of the lichen to its algal host. He admits that there may be osmotic filtration of certain materials from the alga to the lichen, and the like passage of others from the lichen to the alga. However this may be, DANILOV finds the final result to be the absorption of the algae by the lichen hyphae, which enter the algal cells and form dense networks of slender, thin-walled or naked absorbing threads. Although the lichen thallus with its prepared peptones and certain other organic materials is probably a favorable substratum for the algae, yet the lichen is parasitic on the algae, which are killed in large numbers as a result of the parasitism. On the whole the algae thrive better outside the association with the lichen, while the lichen does poorly or dies outright outside the association.—BRUCE FINK.

Sex organs of Phytophthora.—In 1913 PETHYBRIDGE,[8] studying a disease of the potato produced by a phycomycetous fungus which he named *Phytophthora erythroseptica*, observed that on the formation of the sexual organs of this

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[6] DANILOV, A. N., Über das gegenseitige Verhältnis zwischen den Gonidien und dem Pilzkomponten der Flechtensymbiose. Bull. Jard. Imp. Bot. St. Petersb. **10**:33–70. *pls. 3. figs. 9.* 1910.

[7] ———, The relation between the gonidia and the hyphae in lichens. Jour. Botany **56**:169–181. 1918.

[8] PETHYBRIDGE, G. H., On the rotting of potato tubers by a new species of *Phytophthora* having a method of sexual reproduction hitherto undescribed. Sci. Proc. Royal Dublin Soc. **13**:529–565. *pls. 3.* 1913.

fungus the oogonial hypha pushes its way entirely through the antheridium, and, after emerging on the side opposite to the point of entrance, enlarges to form the oogonium. This unusual process, together with the subsequent events in the formation of the oospore, has now been more fully investigated by MURPHY,[9] whose cytological evidence bears out the observations of PETHYBRIDGE. The antheridia and oogonia are found to arise on different branches of the mycelium. During the penetration of the antheridium by the oogonial incept no fusion of the cytoplasm of the two organs occurs. After its emergence the oogonial hypha develops into a more or less spherical multinucleate oogonium whose stalk passes through the antheridium. When the sexual organs have reached their full size, about two-thirds of the nuclei in the antheridium and in the oogonium degenerate. The remaining nuclei in both organs then divide once mitotically and simultaneously. During the division the nuclei of the oogonium are arranged in a hollow sphere, with the exception of one, which remains in the center. Immediately after the division the protoplasm of the oogonium separates into a vacuolate hyaline ooplasm and a denser periplasm. In the oogonium, and probably in the antheridium also, all the nuclei but one degenerate. During this period a prominent receptive papilla protrudes from the base of the oogonium into the antheridium. When the receptive papilla is withdrawn, the fertilization tube grows into the oogonium at the same point and discharges one nucleus and the greater part of the cytoplasm of the antheridium into the oogonium. With the completion of this process most of the periplasm has disappeared and the oospore is surrounded by a thin membrane with the last vestiges of the degenerating nuclei appressed against its outer surface. The fusion of the two nuclei does not take place until the thickened oospore wall has been completed.—H. HASSELBRING.

Action of neutral salts on acid inversion of cane sugar.—LEBERT[10] has studied the action of neutral salts on the acid inversion of cane sugar. His results furnish him a basis for a chemical explanation of certain difficulties sometimes encountered when attempts are made to invert cane sugar by means of weak acids or stronger acids in quantity just sufficient to effect the inversion. Solutions in which it is desired to invert cane sugar are rarely free from neutral salts, especially sodium acetate left in the solution after clearing with lead acetate and removing the excess of lead with sodium carbonate or sulphate. If the hydrolysis is effected by a relatively large quantity of strong acid, as in the Clerget method, the presence of a small amount of salt is of little consequence, since the H ions are in great excess. If organic acids are employed, the presence of their sodium or potassium salts will retard the rate of inversion,

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[9] MURPHY, P. A., The morphology and cytology of the sexual organs of *Phytophthora erythroseptica* Pethyb. Ann. Botany **32**:115–153. *pls. 3.* 1918.

[10] LEBERT, M., Action des sels neutres sur l'inversion du sucre par les acides. Rev. Gen. Botanique **30**:241–244. 1918.

the decrease in the rate depending upon the strength of the acid; the weaker the the acid the greater the inhibiting action of its salt. The action of acetic acid is completely paralyzed by the presence of sodium acetate equivalent to the proportion of the acid. The effect of a salt other than the salt of the acid used for the inversion depends upon the relations established between the acid and the salt. An example would be HCl in the presence of sodium acetate; NaCl and acetic acid are formed. If the acetate is present in sufficient quantity, all of the HCl is replaced by acetic acid, and if the acetate is still in excess, we have inversion by acetic acid in the presence of its sodium salt, in which case the hydrolysis is always inhibited. The author offers a similar explanation for a situation reported by DAVIS and DAISH. They found that 2 per cent citric acid was sufficient to invert a solution of cane sugar by boiling 10 minutes, but it was without effect in the presence of a certain quantity of sodium acetate. The citric acid reacted with the sodium acetate, giving sodium citrate and liberating an equivalent amount of acetic acid, the action of which was paralyzed by its sodium salt still present in the solution.—CHARLES O. APPLEMAN.

Effect of different oxygen pressures on carbohydrate metabolism of sweet potatoes.—The experiments reported by HASSELBRING[11] in this paper were designed primarily to effect a further separation of the various steps in the transformation of starch to sugar in sweet potatoes. For this purpose different oxygen pressures were employed. When the sweet potatoes are killed under a gas pressure of 5 atmospheres, starch hydrolysis is greatly depressed or inhibited. In the living potatoes starch hydrolysis and cane sugar formation proceeded in the absence of oxygen in the same manner as in air or in an atmosphere of oxygen. CRUICKSHANK working with barley seed, and BOYSEN-JENSEN working with germinating barley and peas, found that cane sugar was not formed in the absence of oxygen. These investigators conclude that the presence of oxygen is one of the necessary conditions for cane sugar formation, but since this was not found to be the case with sweet potatoes, the conclusion is not of general applicability.

Anaërobic respiration in sweet potatoes consumes, in a given period of time, a greater quantity of material than is consumed by normal respiration. The energy derived from a given mass of material is less in anaërobic than in normal respiration. These facts, coupled with the observation that cane sugar is formed with equal facility under anaërobic and aërobic conditions, lead the author to believe that his experiments in a general way support the BOYSEN-JENSEN theory that the respiratory processes furnish the energy for the synthesis of cane sugar. In the case of the sweet potato this energy could be furnished by anaërobic respiration.

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[11] HASSELBRING, HEINRICH, Effect of different oxygen pressures on the carbohydrate metabolism of the sweet potato. Jour. Agric. Research 14:273–284. 1918.

Another very interesting fact brought out by the author's work on sweet potatoes is the apparent stability of cane sugar in relation to the respiratory processes in these roots, as cane sugar does not seem to be consumed by either anaërobic or normal respiration.—CHARLES O. APPLEMAN.

Analysis of quantitative variation.—BROTHERTON and BARTLETT[12] have presented the results of a very significant piece of research. The investigation as it stands belongs to the field of plant physiology, but probably it is most significant in the bearing upon certain problems of genetics. Plants of *Phaseolus multiflorus* grown in light and darkness were compared as to length and number of epidermal cells of a given internode. For the physiologist the results may be summarized in the following statement: "The effect of light is that it retards extension of the cells, and that as an indirect result there are fewer secondary divisions, since relatively fewer primary cells enter the range of length within which division takes place." For the geneticist we quote the following: "The mathematical formulation of the results of size inheritance according to the multiple factor hypothesis should be paralleled by a biological analysis, the object of which is the identification of the several factors concerned." Thus size differences may be resolved into number or size of constituent cells or both. "In the investigation of quantitative variations of a hereditary nature it seems likely that the study by the histological method of reactions to the environment and of the obscure reaction known as 'vigor of heterozygosis' will afford a means of correcting for these disturbing factors." It is probably true that heritable size differences express themselves directly in the cells of tissues deeper than the epidermis, and that the change in the epidermis amounts merely to a mechanical response to these forces within. It would probably be advisable, therefore, to carry the analysis to more significant tissues.—MERLE C. COULTER.

Root growth in cuttings.—CURTIS[13] has published an important contribution to the physiology of root formation in cuttings. A number of forms were used, but *Ligustrum ovalifolium* furnished most of the experimental material. Nutrient solutions of the strengths used in culture work with seedlings were found to be distinctly injurious to woody cuttings. Treatments with potassium permanganate resulted in a very marked increase in root growth of various woody cuttings. After discussing several possible explanations for this stimulation, the author concludes that it is most probable that the potassium permanganate increases respiratory activity by catalytically hastening oxidation. It is known that when potassium permanganate comes in contact with organic matter manganese dioxide is precipitated and oxygen is liberated. There was

[12] BROTHERTON, WILBER, and BARTLETT, H. H., Cell measurement as an aid in the analysis of quantitative variation. Amer. Jour. Bot. 5:192–206. 1918.

[13] CURTIS, OTIS F., Stimulation of root growth in cuttings by treatment with chemical compounds. Cornell Univ. Agric. Exper. Sta. Memoir 14:71–138. 1918.

some indication that other inorganic compounds may stimulate root growth in cuttings. The author's work gives further strong evidence that callus and root growth is independent of the rest period and that only the buds assume the resting condition. Immature twigs were caused to absorb cane sugar which increased root development. Mature twigs, however, were but slightly benefited. When the base of cuttings were placed in sugar solution for a short time, the terminal bud of the twig failed to develop in a normal manner and the lower buds formed shoots instead. The author believes that many of the practices commonly followed by greenhouse and nursery men in the propagation of plants by cuttings are explainable on the basis of better aëration. The discussions of the literature are comprehensive and critical.—CHARLES O. APPLEMAN.

Vegetation of Newfoundland.—In contrasting the divergent floras of different parts of Newfoundland, FERNALD[14] bases his explanation of their differences upon the hypothesis that "the presence or absence of varying degrees of available lime or of other bases in the soil is more fundamental in determining plant distribution than are even considerable differences of temperature and humidity."

The calcareous and at the same time the most fertile portion of the island is along the west shore, where the ordinary observer would be surprised to find the indigenous flora of the warmest and most fertile region of the island composed very largely of species of high northern distribution, such as *Juncus triglumis*, *Saxifraga oppositifolia*, *S. aizoides*, *S. caespitosa*, *Salix vestita*, *Dryas integrifolia*, and *Lesquerella arctica*. These FERNALD explains as being from the calcareous habitats of the arctic archipelago and the Canadian Rockies, the lime being hostile to the plants of the siliceous adjacent mainland. The eastern part of the island, the central tundra district, and the southwest corner, in spite of the fact that they are cold, bleak, and barren, are populated mainly by plants of the southern Atlantic coast region, with an addition of some like *Calluna vulgaris* and *Pedicularis sylvatica* from the acid soils of western Europe.

Maps of the distribution of a dozen species give graphic demonstration of the remarkable distribution of some of the more important plants and serve to make the evidence in the support of his hypothesis the more convincing.—GEO. D. FULLER.

Physiological rôle of glucosides in plants.—Continuing his investigations on the physiological rôle of glucosides in plants, COMBES[15] has made the interesting discovery that a given glucoside is not toxic to a plant which naturally

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[14] FERNALD, M. L., The contrast in the floras of eastern and western Newfoundland. Amer. Jour. Bot. **5**:237–247. *pls. 3.* 1918.

[15] COMBES, RAOUL, Recherches biochemiques experimentales sur le rôle physiologique des glucosides ches les vegetaux. Rev. Gen. Botanique **30**:226–237, 245–257. 1918.

produces it, but is very toxic to plants belonging to a species in which the glucoside is not naturally found. The toxic glucoside, when added to Knop's culture medium in which the plants are grown, produces very marked abnormal changes in the morphology of the roots, resulting also in a very stunted growth of tops. It appears that we have here another group of substances, the individuals of which possess a constitution sufficiently characteristic of the species in which they are found that when they are applied to individuals belonging to nonrelated species they produce abnormal responses. The author has not yet found that glucosides will furnish carbohydrate food for plants when they are grown in a carbon dioxide free atmosphere, as has frequently been found to be the case with glucose.

Those wishing to germinate seeds and grow seedlings under aseptic conditions will be interested in the detailed descriptions of the apparatus and procedures employed in growing his plants. An excellent review of the mass of literature on the subject and a survey of glucosides in plants will be found in the earlier papers of this series.—CHARLES O. APPLEMAN.

Physiology of fungi.—DUGGAR, SEVERY, and SCHMITZ[16] have undertaken a study of the comparative nutrient value of some of the decoctions ordinarily used in the preparation of culture media for fungi. The decoctions which were prepared on the basis of 50 gm. of dry matter to a liter of water were made from bean, sugar beet, prune, potato, turnip, cornmeal, apple, mangold, celery, carrot, and salmon. The standard decoctions were employed alone and in combination with sugar and various mineral nutrients. It was found that in their nutrient value the decoctions are very dissimilar for different fungi. The addition of sugar in most cases increases the yield, but the addition of sugar with nitrate and phosphate gives a very much greater yield than the addition of any of these substances alone. It is pointed out also that the standardization of the decoctions on Fuller's scale leaves them differing widely in hydrogen ion concentration. This work brings out the fact that little is really known of the nutrient value of plant decoctions, which it appears are generally deficient in nutrients and require the addition of considerable "fertilizer" to produce the greatest growth of fungi.—H. HASSELBRING.

Maps of rainfall and crop plants.—Among the recent publications of the United States Department of Agriculture there are two at least of decided interest to ecologists and plant geographers. The first is a rainfall map of the United States[17] embodying the data from not less than 3600 stations. The precipitation is given in inches and the map is in 8 shades of blue. An interesting insert map gives the rainfall from April 1 to September 30, and exhibits a

[16] DUGGAR, B. M., SEVERY, J. W., and SCHMITZ, H., Studies in the physiology of fungi. Ann. Mo. Bot. Gard. 4:165–173; 279–288. 1917.

[17] KINCER, JOSEPH B., Atlas of American agriculture. Advance sheet 1: Precipitation. U.S. Dept. Agric. Weather Bur. 1917.

close relationship between the maximum summer rainfall and the grasslands of the country.

The second publication[18] contains relief and precipitation maps of the world and numerous larger and smaller maps showing the agricultural production of all lands. Many other data are contained in the text and in various tables. Several recent papers have successfully related crop possibilities to natural vegetation, but these maps provide material for reversing the process and of relating natural vegetation to areas of crop production.—Geo. D. Fuller.

Seedling of dicotyledons.—Sinnott[19] has made a comparative study of the seedling throughout dicotyledons, in order to distinguish between conservative and variable characters. It is a very timely distinction to emphasize, for the application of the law of recapitulation to variable characters has led to more or less confusion. The number of protoxylem poles is found to be a very variable character. More constant is the relation between the vascular system of the hypocotyl and that of the epicotyl, two main types being recognized. The venation of the cotyledon was found to be very constant; and also an odd number of veins was found to characterize the seedling of all dicotyledons, distinguishing it from that of the gymnosperms. The most conservative character is the structure of the cotyledonary trace.—J. M. C.

Monographs on experimental biology.—The first volume of a series of monographs dealing with experimental biology and general physiology has appeared under the editorship of Jacques Loeb, T. H. Morgan, and W. J. V. Osterhout. The first monograph[20] deals with forced movements, tropisms, and animal conduct. Among the monographs in preparation are "The chromosome theory of heredity" by T. H. Morgan; "Inbreeding and outbreeding; their genetic and sociological significance," by E. M. East and D. F. Jones; "Pure line inheritance," by H. S. Jennings; "The experimental modification of the process of inheritance," by R. Pearl.

This series represents an important event in American science, and deserves the cooperation of the scientific men of the country.—J. M. C.

A new phytopathological journal.—The first number of the *Annals of the Phytopathological Society of Japan* has just appeared, including 5 papers. Some of the papers are in English, and those in Japanese include a summary in English, so that all of them are available for foreign botanists. The contributors to this first number and their titles are as follows: M. Shirai, "On the

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[18] Finch, V. C., and Baker, O. E., Geography of the world's agriculture. 10× 13.5 inches. pp. 149. *figs. 207*. 1917.

[19] Sinnott, E. W., Conservatism and variability in the seedling of dicotyledons. Amer. Jour. Bot. 5:120–130. *figs. 4*. 1918.

[20] Loeb, Jacques, Forced movements, tropisms, and animal conduct. 8vo. pp. 209. *figs. 42*. Philadelphia: J. B. Lippincott Co. 1918. $2.50.

development of plant pathology in Japan"; S. Ito, "A preliminary report on a late blight resistant strain of potato"; T. Hemmi, "Vorlaüfige Mitteilung über eine neue Anthraknose von *Evonymus japonica*"; S. Miura, "On the grain of barley or wheat, infected by smut fungus through the flower"; S. Hori and U. Bokura, "Soy bean cake as a substitute for peptone in the preparation of nutrient media."—J. M. C.

Evolution of maize.—Weatherwax[21] has made a detailed study of the origin of maize, concerning which there has been much discussion. A comparative study of many varieties of maize and related species has led him to the theory that vestigial organs indicate that *Zea*, *Euchlaena*, and *Tripsacum* are of the same structural type, their present peculiarities being due to the suppression of parts present in a primitive ancestor with perfect flowers and one type of inflorescence. The ear of maize is regarded as the homologue of the central spike of the tassel. The prevailing theory that maize is of hybrid origin he regards as untenable, his conclusion being that *Zea* and the other two genera mentioned "have descended independently from a common ancestral form now extinct."—J. M. C.

Ferns of Borneo.—Copeland[22] has brought together in a convenient list the ferns of Borneo, accompanied by analytical keys. The fern flora is very impressive, including 697 recorded species, representing 88 genera. In another paper[23] the same author shows that the riches of the fern flora are far from exhausted, for he describes 43 new species from Borneo, 12 of which are species of *Cyathea*, and also a new genus (*Oreogrammatis*) related to *Polypodium*.—J. M. C.

Bryophytes of Iceland.—Hesselbo[24] has published a rather complete account of the Bryophytes of the island of Iceland. His annotated list shows 93 species of the Hepaticae, 20 of the Sphagnales, and 325 of the Musci. These he further discusses as to their aggregation in communities and their altitudinal and horizontal distribution.—Geo. D. Fuller.

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[21] Weatherwax, Paul, The evolution of maize. Bull. Torr. Bot. Club **45**:309–342. *figs. 36*. 1918.

[22] Copeland, E. B., Keys to the ferns of Borneo. Sarawak Mus. Journ. **2**:287–424. 1917.

[23] ———, New species and a new genus of Borneo ferns. Philipp. Jour. Sci. **12**: 45–65. 1917.

[24] Hesselbo, Aug., The Bryophyta of Iceland. The Botany of Iceland. Ed. by Rosenvinge, L. K., and Warming, Eug. **1**: pt. II. 397–676. 1918.

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EDITED BY

JOHN M. COULTER

With the assistance of other members of the botanical staff of the University of Chicago

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FEBRUARY 1919

BLISTER CANKER OF APPLE TREES; A PHYSIOLOGICAL AND CHEMICAL STUDY

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DEAN H. ROSE

(WITH TEN FIGURES)

Introduction

It is now generally recognized that among the most important problems of plant pathology are those connected with the physiology of diseases whose etiology is already known. It is also recognized that this must be the physiology of the host, of the parasite, and of the two in relation to each other, and, further, that such a comprehensive view of all the factors involved furnishes the only rational approach to an understanding of the principles underlying immunity and disease resistance.

In the present paper are given the results of a physiological study of the destructive disease known as Illinois or blister canker, the etiology of which, including the identity of the causal organism, *Nummularia discreta* (Schw.) Tul., was worked out by HASSELBRING (22) in 1902. The work reported here is a continuation of an earlier investigation by the writer (30) on the oxidase activity of healthy and diseased bark; in addition there is included an account of the catalase activity and microchemical and macrochemical analyses of both kinds of tissues. Further work is planned on the chemistry of the disease, on the rôle of other enzymes than oxidases, and on the physiology of the fungus itself in pure culture.

The work was done in part at the Missouri State Fruit Experiment Station and in part in the Botany Department of the University of Chicago.

Historical

The problem of oxidation by plant and animal tissues or tissue extracts has been studied by many investigators since the time of the pioneer work by SCHÖNBEIN, the discoverer of ozone. An immense literature has accumulated, for reviews of which the reader is referred to publications by CLARK (14), KASTLE (24), BATTELLI and STERN (5), and ATKINS (3). In this paper only those articles will be cited which bear directly on the problem in hand.

That pathological conditions in plants are often accompanied by increased oxidase activity has been shown repeatedly in recent years. WOODS (35) found greater oxidizing power in the chlorotic portions of tobacco leaves affected with mosaic than in the green portions; this has been confirmed by ALLARD (1) and by FREIBERG (20). SORAUER (31, 32) and DOBY (17), working with leaf-roll of potatoes, found oxidase activity greater in diseased tubers than in healthy ones, although the former makes the point that this greater enzyme activity is to be considered a symptom of the disease rather than the cause. BUNZELL (11), working with the curly-dwarf disease of potatoes, showed by an extensive series of tests that "affected plants have a greater oxidase activity than healthy ones of the same age, both in the juice of their tubers and in the juice of their foliage." Similar results were obtained by BUNZELL (10) in work with curly-top of sugar beets. All 4 of these diseases are of the so-called physiological type, and the question is still unsettled for the last 3 whether the increased oxidase activity is the cause of the disease or merely the result of disturbances due to the real but at present unknown cause.

In the case of diseases whose cause is known the oxidase situation seems to be about the same as for those already mentioned. REED (29) found that the juice of apples affected with bitter rot (*Glomerella cingulata*) has greater oxidase activity than that of sound apples. In his previous work the writer (30) found that diseased apple bark shows greater oxidase activity than healthy bark, and is at the same time less acid. This seems to indicate

that the oxidizing power of a tissue bears some relation to its acidity, a relation which was rendered more probable by the fact that, according to titration and indicator tests, the acidity rises in the Bunzell apparatus during the course of an experiment at the same time that oxidation gradually decreases and finally ceases. The suggestion was made, therefore, that "the gradual slowing down of oxidation in the Bunzell apparatus is brought about in part by the accumulation of oxidation products, probably acetic and oxalic acids in the case of pyrogallol, and not by a using up of the oxidase through chemical combination between oxidase and oxidizable substance." The validity of this theory in the light of later investigation will be discussed in the experimental part of this paper.

Experimental

OXIDASE ACTIVITY

EXTRACTS OF FRESH BARK.—An account will first be given of that part of the work done at the Missouri State Fruit Experiment Station. Extracts of fresh Ben Davis bark were used, prepared as follows: limbs were brought in from the orchard, the bark quickly ground in a meat grinder, and water and toluol added in the proportion of 4.25 cc. of toluol for each 100 cc. of water. The mixture was then allowed to extract at 28–30° C. for 1 hour, with frequent stirring, and filtered through filter paper. The proportions of water and toluol used, assuming that the fresh bark contained 50 per cent water, were such as to make the extracts very nearly equivalent to those prepared for the earlier work (30) with dried bark. All data were corrected to the basis of dry weight determined by weighing and drying samples of the ground bark in triplicate to constant weight in a bath at 95–99° C.

Measurement of the amount of oxidation was made by means of the simplified Bunzell apparatus, using 1 cc. of the extract prepared as just described, and either 4 cc. of a 1 per cent solution of pyrogallol, 0.04 gm. of benzidine, or 2 drops (0.025 gm.) of guaiacol; water was added to make the final volume 6 cc. The various combinations of bark, oxidase reagent, and water were run in duplicate.

After the experiment had been set up in the incubator, 1 hour was allowed for the apparatus and solutions to come to a constant temperature. The manometers were then closed and the solutions mixed. No shaking machine was used, but the apparatus holder was tipped back and forth several times whenever a reading was taken. Allowance for temperature variations was made by running with each experiment a blank containing only water and correcting the others by it.

Table I gives the results of two representative experiments, showing the amount of oxidation of the 3 different reagents by

TABLE I

OXIDATION OF PYROGALLOL, BENZIDINE, AND GUAIACOL BY EXTRACTS OF HEALTHY AND DISEASED BARK; MANOMETER READINGS CORRECTED AGAINST APPARATUS CONTAINING ONLY WATER; TEMPERATURE 28–31° C.

DAY OF TEST	EXTRACT OF FRESH BARK						EXTRACT OF DRIED BARK	
	Pyrogallol		Benzidine		Guaiacol		Pyrogallol	
	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
	Sample 38*a*	Sample 38*b*	Sample 41*a*	Sample 41*b*	Sample 38*a*	Sample 38*b*		
1.....	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2.....	0.71	1.62	0.17	0.82	0.02	0.43	0.26	0.57
3.....	1.38	2.46	0.22	1.21	0.11	1.16	0.53	0.94
4.....	1.70	2.50	0.41	1.76	0.21	1.38	0.77	1.20
5.....	1.83	2.50	0.50	2.07	0.27	1.51	0.86	1.36
6.....	.....	.....	.....	.....	.....	.....	1.07	1.50
7.....	2.12	2.66	0.60	2.31	0.32	1.68	.....	.....
8.....	2.20	2.73	0.74	2.52	0.32	1.91	.....	.....
9.....	2.36	2.86	0.77	2.67	0.35	1.98	.....	.....
10.....	2.44	2.96	0.88	2.93	0.42	2.18	1.51	1.94
Ratio..	1.00 to 1.21		1.00 to 3.32		1.00 to 5.19		1.00 to 1.28	

extracts of both healthy and diseased bark. There are included also data from the earlier paper showing the amount of oxidation of pyrogallol by extract of dried bark. The results indicate that for approximately equal amounts of dry matter the dried bark is considerably less active than the fresh (fig. 1). The decrease is probably due to the drying; this is shown more definitely by data to be presented later. It is to be noted that the oxidase activity of diseased bark is definitely greater than that of healthy bark,

although the ratio between the two is greater where benzidine or guaiacol was used as oxidase reagent than where pyrogallol was used. The writer prefers to follow BUNZELL in using the term oxidase activity or oxidizing power rather than "oxidase." Where the latter term occurs in this paper, it is used only for the sake of brevity, with no intent to imply any fixed notion as to the nature of the agent which brings about the oxidation.

Titration and indicator tests on extracts of fresh bark showed the healthy bark to be more acid than the diseased, exactly as had been shown previously in the work with dried bark. No data

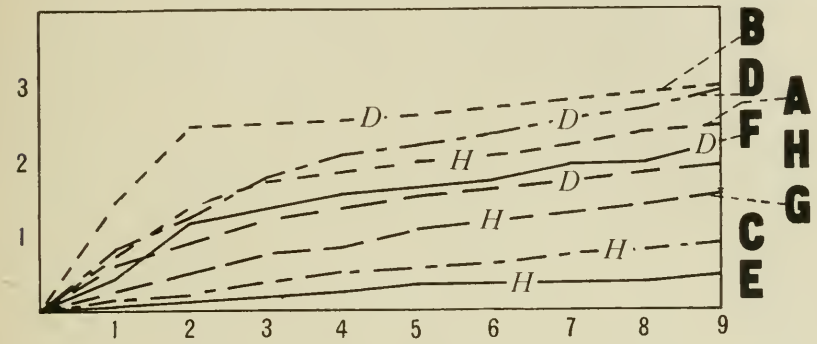

FIG. 1.—Oxidation of pyrogallol, guaiacol, and benzidine by extract of fresh bark, healthy and diseased, and extract of dried bark, healthy and diseased: A, pyrogallol and fresh healthy bark; B, pyrogallol and fresh diseased bark; C, benzidine and fresh healthy bark; D, benzidine and fresh diseased bark; E, guaiacol and fresh healthy bark; F, guaiacol and fresh diseased bark; G, pyrogallol and dried healthy bark; H, pyrogallol and dried diseased bark; *H* = healthy, *D* = diseased.

are given, since the true condition, at least for dried bark, was determined more accurately by means of a potentiometer.

EXTRACTS OF DRIED BARK.—For the work at the University of Chicago bark was used which had been dried at 35–40° C. for 2–3 hours, ground fine enough to go through a 40-mesh sieve, and stored air dry in zinc-capped Mason jars. A few of the experiments were run with oxidases precipitated from an extract of this bark powder, but in most of them the powder itself was used, 0.10 gm. in each apparatus. The reagents tested were pyrogallol and pyrocatechin, 4 cc. of a 1 per cent solution; benzidine 0.05 gm.;

guaiacol 2 drops (0.025 gm.). Tests for any given set of conditions were always run in duplicate, sometimes in triplicate, or even quadruplicate. All experiments were shaken for 3 hours at the rate of 106 complete excursions per minute in a constant temperature chamber provided with a fan driven from the outside, and then allowed to stand for 10–90 hours. Temperature variations were rarely greater than 0.5° during the shaking period, but sometimes amounted to as much as 1.0° afterward, owing to less perfect control when the machinery was not in motion. Corrections for temperature variations were made as before by comparison with a blank containing only water.

Potentiometer measurements were made with a hydrogen electrode like that described by BOVIE (**8**), streaming hydrogen, 3 resistance boxes as described by MICHAELIS (**25**, p. 131), a saturated calomel electrode, a normal element checked against another which had been calibrated by the United States Bureau of Standards, and a Leeds and Northrup dead-beat galvanometer. Hydrogen of high purity from a tank of the compressed gas was run through an electrically heated combustion tube containing platinized asbestos and then through the hydrogen electrode tube. The latter, together with the capillary from the calomel electrode, projected through a rubber stopper into the vessel containing the solution to be tested. Escape of hydrogen was provided for by a third opening in the stopper. An error was undoubtedly introduced here, due to displacement of CO_2 from the solution, in cases where the hydrogen ion concentration was less than 10^{-5} (MICHAELIS, pp. 142–144), but since the only solutions showing this slight degree of acidity were mixtures of bark, water, and pyrogallol for determination of hydrogen ion concentration before any oxidation had taken place, and since all others were found to be more acid, the error is probably negligible. It could have been avoided entirely by using a Hasselbalch shaking electrode had it and the time for using it been available.

Among the first experiments run was one designed to test fully the oxidase activity of healthy and diseased bark when pyrogallol was used as the oxidizing substance. The results, given in table II, are the average of 5 closely agreeing determinations. These results

agree well with those obtained without a shaking machine in showing considerably greater oxidation by diseased than by healthy bark. The ratio between the two, 1.00:2.19, is larger than that found previously (1.00:1.28), the difference probably being due to differences in drying or possibly to the shaking itself.

TABLE II

OXIDATION OF PYROGALLOL BY HEALTHY AND DISEASED APPLE BARK; SAMPLES 3 AND 4; TEMPERATURE 27 C±1.7° C.

TIME OF READING	MANOMETER READINGS, EXPRESSED IN CM. OF MERCURY, CORRECTED AGAINST BLANK CONTAINING ONLY WATER		TIME OF READING	MANOMETER READINGS, EXPRESSED IN CM. OF MERCURY, CORRECTED AGAINST BLANK CONTAINING ONLY WATER	
	Healthy	Diseased		Healthy	Diseased
March 19					
2:45 P.M.	0.0	0.0	March 19	0.48	1.25
3:00	0.0	0.23	4:45 P.M.	0.53	1.33
3:15	0.10	0.48	5:00	0.61	1.45
3:30	0.16	0.65	5:15	0.64	1.57
3:45	0.23	0.80	5:30	0.59	1.49
4:00	0.31	0.92	5:55		
4:15	0.41	1.05	March 20	1.10	2.41
4:30	0.45	1.11	8:30 A.M.		

In table III are summarized the results of an experiment to test the oxidizing power of both diseased and healthy bark on pyrocatechin, guaiacol, and benzidine.

A comparison of the figures in table III with those in tables I and II shows that diseased bark causes greater oxidation of pyrogallol, pyrocatechin, benzidine, and guaiacol than does healthy bark, and that both tissues cause greater oxidation of the first two reagents than of the last two. It is further shown by tables I and III that the amount of oxidation increases slowly for several days; in fact table III shows that it is practically doubled for all the combinations, except those containing pyrocatechin, during the 64-hour period following the 3 hours' shaking. This fact of an increase of oxidation on standing was observed to a greater or less degree with most of the bark material used in this work, and is in direct contradiction to BUNZELL'S explicit and repeated statement that oxidation in his apparatus comes to a definite end after 3 or 4 hours' shaking. The only exceptions the writer has

noted were in those cases where the bark powder showed low oxidase activity to begin with, possibly due to injury of the "oxidase" during drying.

TABLE III

OXIDATION OF PYROCATECHIN, GUAIACOL, AND BENZIDINE BY HEALTHY AND DISEASED BARK; TEMPERATURE 29.4–29.7° C.

TIME OF READING	HEALTHY			DISEASED		
	Benzidine	Guaiacol	Pyrocatechin	Benzidine	Guaiacol	Pyrocatechin
June 8, 1:30 P.M....	0.0	0.0	0.0	0.0	0.0	0.0
4:30 after shaking 3 hours..	0.08	0.33	1.13	0.65	0.75	3.77
June 9, 8:10 A.M...	0.25	0.35	1.45	0.80	1.00	4.35
2:20 P.M...	0.38	0.48	1.65	0.98	1.07	4.55
" 10, 9:15 A.M...	0.40	0.55	1.85	1.27	1.20	4.87
" 11, 8:20 A.M...	0.65	0.65	2.12	1.45	1.47	5.12

That the rate and temperature of drying have an effect on the oxidase activity as well as on the hydrogen ion concentration is clearly shown in table IV.

TABLE IV

EFFECT OF RATE AND TEMPERATURE OF DRYING UPON OXIDASE ACTIVITY AND HYDROGEN ION CONCENTRATION OF HEALTHY AND DISEASED APPLE BARK

SAMPLE	OXIDATION			INITIAL P_H	TEMPERATURE AND DURATION OF DRYING	DEGREE OF BROWNING
	After shaking 3 hours	After standing 10 hours	After standing 15 hours			
4 diseased..	1.49	2.33	2.78	5.61*	40°, 2 hours	Slight
6 " ..	2.25	2.42	.....	5.45	40°, 2	Slight
2 " ..	1.58	1.60	.....	5.16	40°, 4	Much
3 healthy...	0.59	0.80	1.23	5.15	40°, 2	Very little
5 " ...	1.07	1.12	.....	5.04	40°, 2	Slight
5a " ...	0.35	0.35	.....	5.00	50°, 2	Very little
1 " ...	0.62	0.72	.....	4.80	35°, 4	Much

* This figure is the negative logarithmic exponent of 10 where the whole expression $10^{=5.61}$ is a measure of the hydrogen ion concentration in the solution. The larger it is, therefore, the smaller the hydrogen ion concentration it expresses. In this particular case it can be written 2.454×10^{-6} (6.00 − 5.61 = 0.39. Antilog 0.39 = 2.454). In the amplified form this becomes 0.00000 2454 (normal).

Samples 1, 2, 5, 5a, and 6 were all run in one experiment. Oxidation data for samples 3 and 4 are taken from table II and from another experiment not recorded in this paper. Samples 5 and 5a were parts of the same lot of ground bark but received

different treatments as shown. The results show that oxidase activity is much reduced by drying at 35–40° for 4 hours (sample 1, healthy; sample 2, diseased), or at 50° for 2 hours (sample 5a, healthy).

HYDROGEN ION CONCENTRATION.—Hydrogen ion determinations on mixtures of bark and water and of bark, water, and pyrogallol, used in the same proportions as in the oxidase apparatus, showed that pyrogallol has no effect on the reaction. It was found possible to get constant initial readings on all mixtures containing healthy bark and pyrogallol in 30–45 minutes; the same period sufficed for mixtures containing diseased bark and pyrogallol after they had been shaken in the oxidase apparatus, but not for similar mixtures freshly made up and not shaken. In these cases the potential increased slowly for an hour or two from about $P_H = 5.60$ to $P_H = 5.40$, but never reached the figure given by healthy bark.

CULPEPPER, FOSTER, and CALDWELL (16), working with normal and diseased Red Astrachan apples, state that when titrations were made on fruit pulp suspended in water "the diffusion of acids out of the tissues continues for many hours and at slower rates in diseased than in normal fruits," but in the light of the following results the writer is inclined to think this increase of acidity was due to oxidation going on in the solutions, and not to diffusion of acids out from the tissues.

TABLE V

CORRELATION BETWEEN OXIDASE ACTIVITY AND HYDROGEN ION CONCENTRATION OF MIXTURES CONTAINING PYROGALLOL, WATER, AND EITHER HEALTHY OR DISEASED BARK; TEMPERATURE 29–30.5° C.

STAGE OF EXPERIMENT	HEALTHY		DISEASED	
	Oxidation	P_H	Oxidation	P_H
Before shaking	0.00	5.15	0.00	5.61
After shaking 3 hours	0.82	.....	2.28	.....
After standing 15 hours	1.10	4.82	2.59	4.89
" " 48 "	2.00	.....	4.07	.....
" " 64 "	2.90	4.29	4.96	4.29

INCREASE IN HYDROGEN ION CONCENTRATION DURING OXIDATION.—Experiments designed to test more fully the theory that oxidation causes an increase in acidity are summarized in table V.

It is clear from table V that oxidation in these mixtures is accompanied by a marked increase in hydrogen ion concentration, and the conclusion certainly seems justified that there is a causal relation between the two. It is also seen that when oxidation comes to an end, both mixtures have the same reaction, $P_H = 4.29$, a condition suggesting that at this point the hydrogen ion is the limiting factor.

BUNZELL (12) and REED (28) have studied the effect of hydrogen ion concentration on oxidation, but apparently neither of them has realized that it might increase during the oxidation process (30). They apparently assume that the hydrogen ion concentration established at the beginning of an experiment remains constant until the end, whereas the results given show that in these cases it increased as long as the oxidation continued.

In order to discover, if possible, what relation exists between oxidation and hydrogen ion concentration in the oxidase apparatus, further experiments were tried with mixtures of bark, dry pyrogallol, and, instead of water, 5 cc. of buffer solutions containing various amounts of N/10 sodium acetate and either N/10 or N/100 acetic acid. The initial reactions of these mixtures (before shaking) and of the buffers alone are given in table VI and shown graphically in fig. 2.

TABLE VI

REACTION, P_H, OF BUFFER SOLUTIONS AND MIXTURES OF BUFFER SOLUTIONS, BARK, AND PYROGALLOL

Solution	1	2	3	4	5	6	7	8	9
Buffer alone.....	6.02	5.73	5.41	5.17	4.80	4.53	4.21	3.90	3.61
Buffer and healthy bark and pyrogallol.....	5.59	5.52	5.36	5.15	4.85	4.58	4.24	3.98	3.61
Buffer and diseased bark and pyrogallol.....	5.76	5.70	5.50	5.31	5.00	4.61	4.39	4.08	3.73

Graphs B and C in fig. 2 show that while diseased bark absorbs H^+ ions to about the same extent as the healthy, the latter absorbs more OH^- ions; that is, its titration acidity is greater, which is exactly the condition found by titration with N/20 sodium hydroxide (30). The P_H values at points where B and C cross A

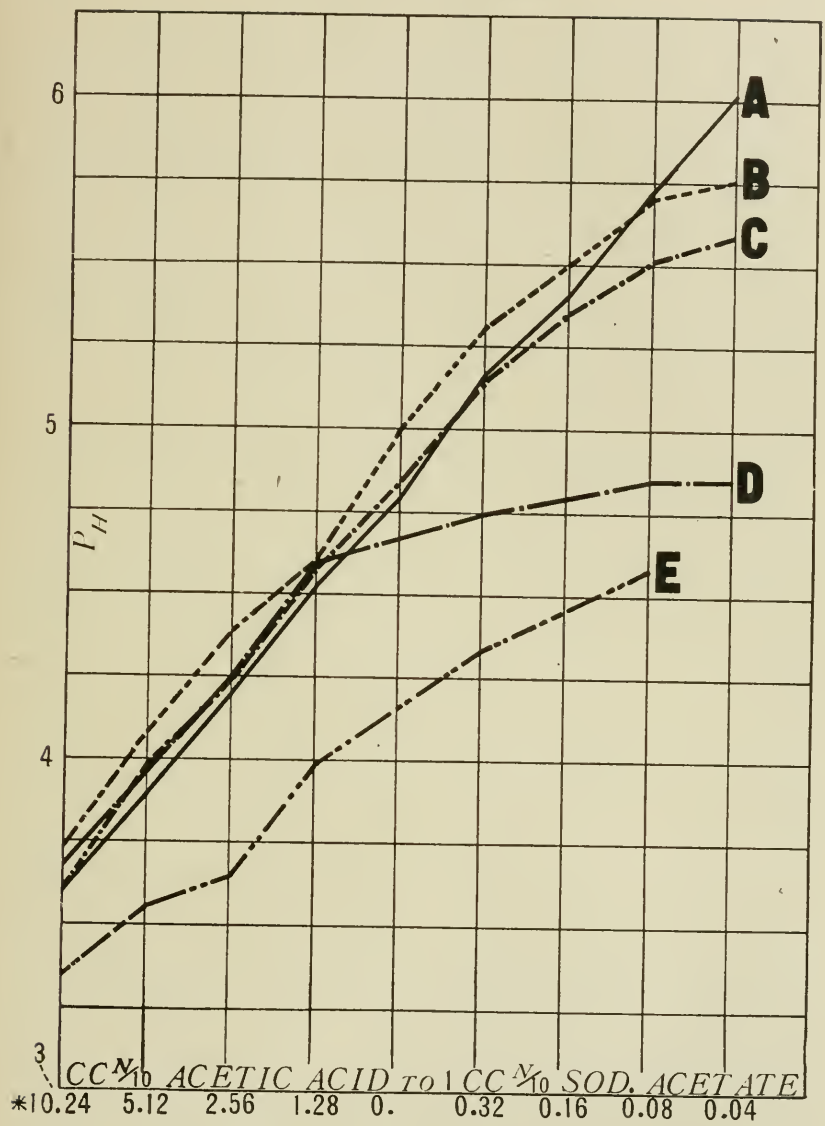

FIG. 2.—P_H of mixtures of bark, pyrogallol, and various buffer solutions before and after oxidation had ceased: A, P_H of buffer solutions; B, P_H of mixtures of buffer solutions, pyrogallol, and diseased bark before oxidation; C, P_H of mixtures of buffer solutions, pyrogallol, and healthy bark before oxidation; D, P_H of mixtures of buffer solutions, pyrogallol, and diseased bark after oxidation; E, P_H of mixtures of buffer solutions, pyrogallol, and healthy bark after oxidation. *Only acetic acid used here.

(healthy bark about 5.10, diseased about 5.65) agree well with those determined without the buffer (P_H healthy = 5.15, diseased = 5.61); the latter are taken, therefore, to represent practically the actual acidity in each case. This is based on the assumption that if the acidity of a buffer solution is the same as that of a mixture of bark, pyrogallol, and water, no change in acidity will take place when the buffer is used instead of water.

EFFECT OF BUFFER SOLUTIONS.—The oxidations brought about by mixtures of bark, pyrogallol, and the various buffer solutions are given in table VII, together with the initial P_H of these mixtures and their P_H after oxidation had practically ceased.

TABLE VII

OXIDATION BY MIXTURES OF BARK AND PYROGALLOL WITH VARIOUS BUFFER SOLUTIONS; TEMPERATURE 29–30° C.

BUFFER SOLUTION	HEALTHY			DISEASED		
	Oxidation	Initial P_H	Final P_H	Oxidation	Initial P_H	Final P_H
1	.....	5.59	.....	4.68	5.76	4.85
2	1.68	5.52	4.58	4.78	5.70	4.85
3	.....	5.36	.....	.....	5.50	.....
4	2.15	5.15	4.34	4.36	5.31	4.75
5	.....	4.85	.....	.....	5.00	.....
6	1.95	4.58	3.98	4.48	4.61	4.60
7	1.80	4.24	3.65	4.12	4.39	4.25
8	1.55	3.98	3.56	.....	4.08	.....
9	0.53	3.61	3.35	1.82	3.73	3.68
Check	2.22	5.15	4.29	4.27	5.61	4.29

The principal fact shown by the results in table VII is that the P_H (4.29) reached by mixtures of pyrogallol, water, and either healthy or diseased bark when oxidation comes to an end is not sufficient to inhibit oxidation when the mixture has that P_H value to begin with; in fact, a greater degree of acidity does not inhibit entirely, since a healthy bark mixture with an initial P_H of 3.61 gave an oxidation (a mercury rise) of 0.53 cm., and a diseased bark mixture with an initial P_H of 3.78 gave an oxidation of 1.82 cm. The check, bark, pyrogallol, and water gave, in the former case, 2.22 cm. mercury rise, and in the latter 4.27 cm.

It might seem from this that the acidity brought about in mixtures of bark, pyrogallol, and water is not the factor which

brings oxidation to an end. It seems more reasonable to suppose, however, that the time factor is of importance here; that is, that an acidity of $P_H = 4.29$ is more effective when reached gradually than when established as a starting point. Looking at the situation from another angle, we may say that inhibition is total if the initial hydrogen ion concentration is high enough, but will be only partial if the concentration is lower; but since partial inhibition means some oxidation, which in itself increases acidity, the process in time necessarily comes to an end. The hydrogen ion concentration at that point will depend on what it was in the beginning, but will never be equal to that which causes total inhibition.

That this theory fits the facts is shown by table VII. Oxidation took place in all the mixtures, the amount depending on the initial hydrogen ion concentration, except where diseased bark was used with buffer no. 4. Acidity increased in all the mixtures but one, diseased bark with buffer no. 6 (see tables VI and VII). The increase in acidity is shown graphically in fig. 2. It is unexpectedly small for diseased bark except where the 3 most alkaline buffers were used, a condition which suggests the need of further experiments.

In figs. 3 and 4 are shown graphically the oxidation data given in table VII, representing the final amounts of oxidation for each set of tests (healthy and diseased bark with the different buffer solutions). In addition there are shown graphs for several earlier stages in each experiment. These graphs show that below 1×10^{-4} ($P_H = 4$) for healthy bark, and 2.5×10^{-5} ($P_H = 4.39$) for diseased bark, oxidation drops rapidly as acidity increases. Above these points the changes are not so marked. The hydrogen ion concentration for total inhibition, estimated by extrapolation to the base line, lies between 3.55 and 3.80×10^{-4} for healthy and between 3.55 and 4.27×10^{-4} for diseased bark. All these figures closely approximate those found by BUNZELL (**12**) for potato oxidase, 2.1–2.8×10^{-4}, and by REED (**28**) for apple oxidase, 5.0–7.0×10^{-4}.

The results given in table VII show that hydrogen ion concentration is not the only factor effective in controlling oxidation in the apparatus, and consequently that the lower hydrogen ion concentration of diseased bark cannot account entirely for its

greater oxidizing power. For example, when both kinds of bark were brought to approximately the same hydrogen ion concentration by buffer no. 6, the final amount of oxidation (mercury rise) for healthy bark was 1.95 and for diseased 4.48, the final P_H 3.98 and 4.60 respectively. The total oxidase activity of the diseased plant is the joint oxidase activity of the host and parasite, while

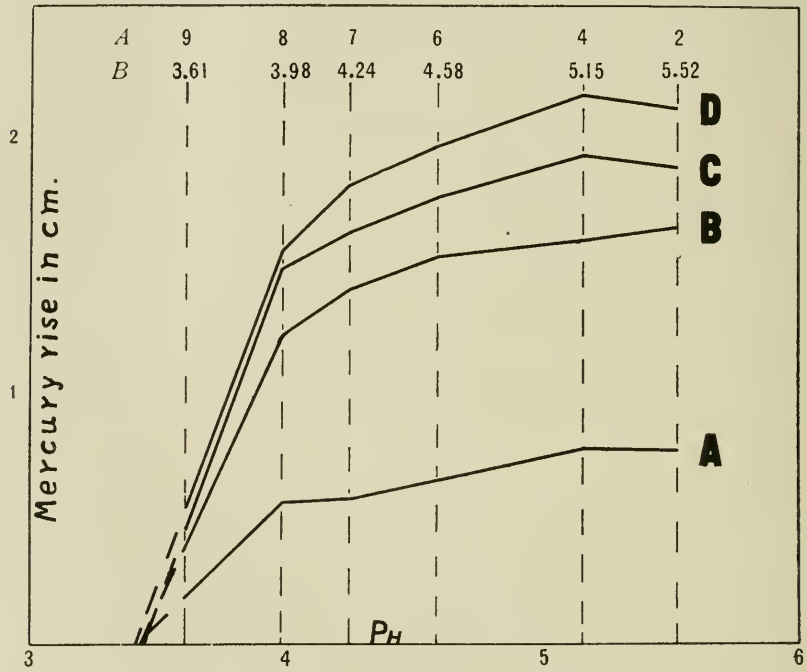

FIG. 3.—Oxidation of mixtures of healthy bark, pyrogallol, and various buffer solutions: A, after 3 hours; B, after 22 hours (19 hours without shaking); C, after 29 hours; D, after 48 hours; *A*, bark, pyrogallol, and buffer solutions as indicated by numbers; *B*, initial P_H; points of plotting marked by vertical broken lines.

the oxidase activity of the healthy plant is that of the host alone. This may account in part for the difference both in rate of activity and in the P_H concentration at the time the action ceases.

NATURE OF EQUILIBRIUM REACHED.—BUNZELL (13), in experiments with potato peel powder, has obtained what he considers evidence that "the activity of the plant powder is not paralyzed by the products formed in the course of the reaction." He found

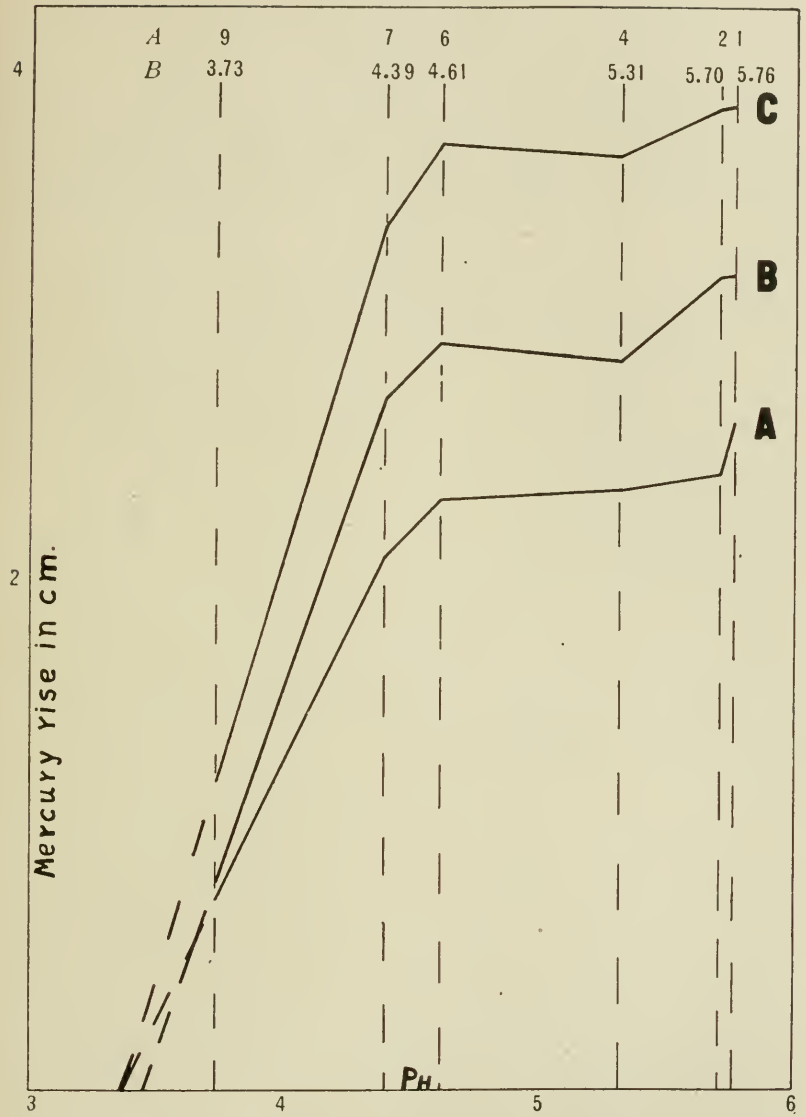

FIG. 4.—Oxidation by mixtures of diseased bark, pyrogallol, and various buffer solutions: A, after 23 hours (shaken 2 hours of this time); B, after 45.5 hours; C, after 69.5 hours; *A* and *B* as in fig. 3.

that by adding a second portion of the powder to the apparatus in which oxidation by the first portion had ceased he could cause a further increase in oxidation, the amount of increase varying with the oxidase reagent used. The writer has found a similar increase in oxidation when more oxidase reagent is added, after oxidation ceases. . The results of an experiment of this kind are summarized in table VIII. Results are given beginning with the

TABLE VIII

SUMMARY OF RESULTS FROM AN EXPERIMENT TO TEST EFFECT OF ADDING FRESH SUPPLY OF OXIDASE REAGENT.

Experiment	Stage of experiment	Increase in oxidation (cm. of mercury rise)			
		8	9	10	11
Effect of adding 7 and 4 drops 1 per cent benzidine to apparatus 10 and 11 on ninth day, 8 and 9 as checks	9th to 11th	0.00	0.02	0.49	0.19
Effect of adding 10 drops 1 per cent benzidine to apparatus 10 and 11 on eleventh day, 8 and 9 as checks	11th to 14th	0.17	0.23	1.16	0.71
Total effect of 1 per cent benzidine, 8 as check	9th to 26th	0.47	.....	1.95	1.34
Effect of adding 0.06 gm. of pyrogallol to 8 on twenty-sixth day	26th to 41st	1.53	.....	.....	.....
8 as check	9th to 26th	0.47	.....	.....	.....
Effect of adding 0.06 gm. benzidine to 9 on fourteenth day, 8 as check	14th to 21st	0.19	0.74	.....	.....
Effect of adding 10 drops absolute alcohol to 9 on twenty-first day, 8 as check	21st to 26th	0.11	0.68	.....	.....

ninth day of the experiment. Up to that time oxidation in all 4 of the tubes was practically the same, the average being 3.12 (cm. of mercury rise). Alcohol was used at the beginning of the experiment to discover whether it has an inhibiting effect on oxidation, and later, when solid benzidine was added, to bring the benzidine into solution more rapidly. The results show that, in the small quantities used, the alcohol had no inhibiting effect (table VIII, ninth day, apparatus 10 and 11), and probably did bring the benzidine into solution (twenty-first–forty-first day, apparatus 9).

The most important fact shown by these results is that after oxidation had practically ended, the addition of more oxidase

reagent was followed by a marked increase in oxidation. For example, in table VIII it is seen that from the ninth to the twenty-sixth day oxidation in apparatus 8, containing pyrogallol and bark extract, showed an increase of only 0.47 cm., while tubes 10 and 11, also containing pyrogallol and bark extract to which benzidine solution was added later, showed an increase of 1.95 and 1.34 cm. respectively. Equally marked excess over the check was obtained when solid pyrogallol or solid benzidine was added. One might infer that the oxygen admitted, when the tubes were opened to introduce reagents, increased oxidation, but this effect could hardly account for the difference observed. BUNZELL states that exhaustion of oxygen is not the limiting factor, and experiments by the writer have shown that, when a fresh oxygen supply is allowed to enter the apparatus, the subsequent increase in oxidation is small.

The fact that after oxidation ends it can be started afresh by the addition of fresh plant material or of fresh oxidase reagent suggests that the equilibrium reached is a false one, like the third case described by HÖBER (**23**, p. 671), in which a reaction product of the catalytic reaction brings about equilibrium by an inactivation of the catalyzer. A test for this condition according to HÖBER is that reaction begins again when more catalyzer is added, as in the case of the hydrolysis of amygdalin by emulsin. The similarity between the two reactions, however, does not prove that the oxidation catalyst is an enzyme, for it may be non-enzymic in nature and still be inactivated by the products of the catalytic reaction.

An idea of the nature of the oxidase reaction was obtained by testing some of the data by the formula for unimolecular reaction, $k = \frac{1}{t} \log. \frac{a}{a-x}$. In these calculations the total amount of oxidation (mercury rise) at the end of the shaking period was assumed for the value of a, and the amount of oxidation at the end of each 15-minute interval for the value of x. The figures which should be used, of course, are the total amount of pyrogallol at the beginning of the experiment and the amount oxidized at the end of each 15-minute interval, but such figures would be difficult to obtain. The writer sees no reason why the values used for a and x do not truly represent the course of the reduction.

In most cases the values of k given in these tables are fairly constant and may be considered a strong indication that the oxidase reaction is unimolecular. In table XI, column 3, table XII,

TABLE IX

HEALTHY BARK AND PYROGALLOL

t (min.)	x (mercury rise in cm.)	$a-x$	$k=\frac{1}{t}\log.\frac{a}{a-x}$
15	0.14	0.72	[0.00514
30	0.19	0.67	0.00361
45	0.24	0.62	0.00315
60	0.34	0.52	0.00365
75	0.44	0.42	*[0.00415
90	0.49	0.37	0.00407]
105	0.54	0.32	0.00409
120	0.63	0.23	0.00477
135	0.68	0.18	0.00503
150	0.74	0.12	0.00570]
165	0.79	0.07	0.00660
180	0.86	.....	.....
Mean	.....	.....	0.00433

* Brackets in this and following tables indicate those values of k which were considered in calculating the mean.

TABLE X

DISEASED BARK AND PYROGALLOL

t (min.)	x (mercury rise in cm.)	$a-x$	$k=\frac{1}{t}\log.\frac{a}{a-x}$
15	0.13	1.25	0.00286
30	0.30	1.08	[0.00355
45	0.50	0.88	0.00434
60	0.65	0.73	0.00461
75	0.72	0.66	0.00427
90	0.85	0.53	0.00461]
105	0.99	0.39	0.00522
120	1.04	0.34	0.00507
135	1.15	0.23	0.00576
150	1.18	0.20	0.00559]
165	1.25	0.13	0.00621
180	1.38	.....	.....
Mean	.....	.....	0.00478

column 1, and table XIV, column 1, the values for k show a gradual increase throughout the experiment, and can scarcely be taken to indicate a unimolecular reaction. Table XII, column 1, however, is checked by tables IX and XII, column 3, the mean value

of k being nearly the same in all 3 cases, although it is doubtful whether a mean for table XII, column 1, is really significant.

TABLE XI

VALUES OF k CALCULATED FROM DATA OBTAINED IN EXPERIMENTS WITH APPLE BARK, K_2CO_3, AND PYROGALLOL

t (min)	k			
	K_2CO_3 and pyrogallol	K_2CO_3 and pyrogallol	Healthy bark, K_2CO_3, and pyrogallol	Diseased bark, KC_2O_3, and pyrogallol
15	0.00747	0.00552	0.00114	0.00525
30	0.00776	0.00803	0.00380	0.00600
45	0.00774	0.00773	0.00431	0.00615
60	0.00785	0.00786	0.00368	0.00604
75	0.00808	0.00819	0.00472	0.00606
90	0.00767	0.00750	0.00492	0.00628
105	0.00765	0.00709	0.00514	0.00642
120	0.00786	0.00890	0.00470	0.00657
135	0.00811	0.00941	0.00633	0.00700
150	0.00768	0.00947	0.00663	0.00678
165	0.00805	0.00936	0.00692	0.00862
180	.....	.....	.....	.....
Mean..	0.00781	0.00834	0.00482	0.00635

TABLE XII

VALUES OF k CALCULATED FROM DATA OBTAINED IN EXPERIMENTS WITH APPLE BARK, PYROGALLOL, AND PYROCATECHIN

t (min.)	k			
	Healthy bark and pyrogallol	Diseased bark and pyrogallol	Healthy bark and pyrocatechin	Diseased bark and pyrocatechin
15	.....	0.00458	0.00502	0.00483
30	0.00246	0.00528	0.00429	0.00494
45	0.00277	0.00515	0.00357	0.00510
60	0.00322	0.00515	0.00346	0.00488
75	0.00383	0.00510	0.00416	0.00526
90	0.00493	0.00530	0.00433	0.00536
105	0.00460	0.00507	0.00426	0.00553
120	0.00501	0.00575	0.00434	0.00584
135	0.00584	0.00604	0.00483	0.00613
150	0.00886	0.00744	0.00548	0.00690
165	.....	.....	0.00590	0.00866
180	.....	.....	.....	.....
Mean..	0.00430	0.00527	0.00451	0.00521

Confirmation of the results with apple bark is found in table XIII and table XIV, column 2, based on data obtained by

BUNZELL (9, 13) with tulip tree leaves and with potatoes, although the mean value of k in all 3 cases is much larger than that found for bark. Attention has already been called to the fact that the data in table XIV, column 1 (also from BUNZELL's work), fail to fit the equation for a unimolecular reaction. The fact of a marked rise

TABLE XIII

VALUES OF k CALCULATED FROM DATA PUBLISHED BY BUNZELL (9) FOR POTATO JUICE AND PYROGALLOL

t (min)	k*	t (min)	k†
10	0.0315	10	0.0246
20	0.0266	30	0.0277
30	0.0240	45	0.0199
40	0.0216	60	0.0168
50	0.0244	75	0.0174
60	0.0277	90	0.0233
70	0.0255	105	.....
80	0.0283	Mean	0.0208
90	.....		
Mean	0.0262		

* **23**, p. 29, table VII, columns 1 and 4.
† **23**, p. 26, table II, columns 5 and 7.

TABLE XIV

VALUES OF k CALCULATED FROM DATA PUBLISHED BY BUNZELL (13)

t (min.)	k		t (min.)	k	
	Spinach leaves and para-cresol	Tulip tree leaves and phlorhizin		Spinach leaves and para-cresol	Tulip tree leaves and phlorhizin
15	0.00374	0.0124	90	0.01018	.....
30	0.00654	0.0119	105	.....	.....
45	0.00640	0.0133	120	.....	.....
60	0.00940	0.0137	135	.....	.....
75	0.01092	.....	Mean	.....	0.0137

in the value of k toward the end of the experiments with bark may mean that at that point the "oxidase" oxidizes not *constant fractions* but *constant weights* of pyrogallol in a given time (PHILIP **27**, p. 295). The data at hand, however, are insufficient for a verification of this hypothesis.

A unimolecular reaction is one in which the concentration of only one substance is changed. If oxidation of pyrogallol by plant material in the oxidase apparatus be such a reaction, the substance whose concentration is changed is pyrogallol. The "oxidase" then appears as the catalyst, its concentration remaining unchanged during the course of the reaction. Even at that it is not necessarily proved to be an enzyme, since the linear relationship between time and amount of change is also shown in the oxidation of pyrogallol by potassium carbonate.

Effect of adding protective colloids.—Bayliss (6) and Perrin (26) have suggested that the oxidizing enzyme is an active form of the colloidal hydroxide of manganese, iron, or copper, kept in this active state by an emulsion colloid such as gum or albumin, acting as a protective colloid. Tables XV and XVI show the effects of additions of gelatine and gum arabic. Table XV shows that 0.2 per cent gelatine increases considerably the oxidation by healthy bark and only slightly that by diseased bark. Three other experiments with pyrogallol and 2 with pyrocatechin with 0.2 per cent gelatine added showed similar results. The use of 0.8 per cent gelatine with pyrogallol also showed a similar effect. Both 0.2 and 0.8 per cent gum arabic had little or no effect on healthy bark and a slight accelerating effect on diseased bark.

TABLE XV

Effect of 0.2 per cent gelatine on oxidation of pyrogallol by healthy and diseased bark; temperature 22–24° C.

Time of reading	Healthy		Diseased	
	Without gelatine	With gelatine	Without gelatine	With gelatine
May 18, 7:03 P.M.	0.0	0.0	0.0	0.0
10:03 P.M. after shaking 3 hours.	0.77	0.81	2.22	2.24
" 19, 8:10 A.M.	0.94	1.32	2.40	2.54
" 20, 9:35 A.M.	1.37	2.26	2.88	3.00
" 21, 8:15 A.M.	1.65	2.74	3.17	3.27

Since gelatine is amphoteric, one might infer that it or its splitting products act as buffers, thus reducing the rate of increase of the hydrogen ion concentration with progress of the oxidation

(fig. 5). Table XVII, however, shows that gelatine has little effect on the hydrogen ion concentration of oxidizing mixtures of either healthy or diseased bark.

PRECIPITATED OXIDASES.—Experiments were run using precipitated "oxidases," prepared as follows: 2 gm. of bark were allowed to extract with 10 cc. of water and 5 drops of toluol for 1 hour; the extract was then squeezed through moist cheesecloth on to coarse filter paper, the beaker washed with five 1 cc. portions of water and the filter paper finally with two more; 50 cc. of 95 per

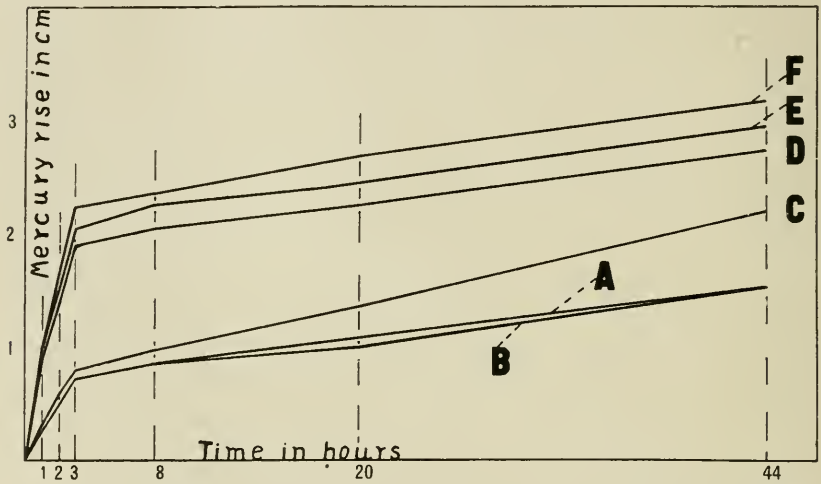

FIG. 5.—Effect of 0.8 per cent gum arabic and 0.8 per cent gelatine on oxidation of pyrogallol by healthy and diseased bark: A, healthy bark; B, healthy bark and gum arabic; C, healthy bark and gelatine; D, diseased bark; E, diseased bark and gum arabic; F, diseased bark and gelatine.

cent alcohol were then added to the filtrate (concentration of alcohol about 70 per cent), the whole allowed to stand for 10 minutes and the flocculent precipitate collected on a hard filter by gentle suction with a filter pump; 150 cc. more alcohol were then added to the filtrate (concentration of alcohol now about 90 per cent) and the whole allowed to stand for 1 hour, since precipitation was slow, before collecting this second fraction on the filter with the first. The precipitate from diseased bark was much browner than that from healthy bark. Whether this bears any relation to its greater oxidase activity is not known.

For tests in the oxidase apparatus the combined precipitates were dissolved in 20 cc. of water, and 2 cc. of this solution containing the precipitate obtained from 0.1 gm. of bark was put in each apparatus together with the usual amounts of pyrogallol and water.

TABLE XVI

EFFECT OF 0.2 PER CENT GUM ARABIC, 0.8 PER CENT GUM ARABIC, AND 0.8 PER CENT GELATINE ON OXIDATION OF PYROGALLOL BY HEALTHY AND DISEASED BARK; TEMPERATURE 21–23° C.

TIME OF READING	HEALTHY				DISEASED			
	No addition	Gelatine 0.8 per cent	Gum arabic		No addition	Gelatine 0.8 per cent	Gum arabic	
			0.2 per cent	0.8 per cent			0.2 per cent	0.8 per cent
At beginning....	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
After shaking 3 hours.....	0.70	0.78	0.65	0.71	1.88	2.23	2.19	2.05
After 18.5 hours	1.03	1.34	1.00	1.01	2.36	2.69	2.71	2.46
After 42 hours..	1.50	2.20	.....	1.54	2.73	3.18	.....	2.95
Average of.....	2	.....	.....	.....	2	.....	.....	.....

In table XVIII are given results showing the oxidizing power of these solutions, with and without gelatine (fig. 6).

The relation observed with bark powder still holds here, that diseased material is more active than healthy. On the other hand, gelatine increases oxidation by the precipitate from extract of

TABLE XVII

REACTION OF MIXTURES OF BARK AND PYROGALLOL WITH GELATINE (0.2 PER CENT) AND WITHOUT AT VARIOUS STAGES OF OXIDATION PROCESS

TIME OF READING	HEALTHY		DISEASED	
	Without gelatine	With gelatine	Without gelatine	With gelatine
P_H Initial.....	5.15	5.15	5.61	5.60
P_H After 15 hours.....	4.82	4.84	4.89	4.86
P_H After 64 hours.....	4.29	4.35	4.29	4.52

diseased bark, but is without marked effect on that from healthy bark, the reverse of the condition found when bark powder was used.

There were indications in the preliminary work that the alcoholic precipitate from bark extract was easily separated into 2

fractions, hence it seemed worth while to collect these separately. This was done for both healthy and diseased tissue and gave

TABLE XVIII

OXIDATION OF PYROGALLOL BY AQUEOUS SOLUTIONS OF PRECIPITATED OXIDASE FROM HEALTHY AND DISEASED BARK, WITH AND WITHOUT GELATINE; TEMPERATURE 29.3–30.3° C.

TIME OF READING	HEALTHY		DISEASED	
	Without gelatine	With gelatine	Without gelatine	With gelatine
June 28, 4:35 P.M.	0.0	0.0	0.0	0.0
" 29, 8:45 A.M.	0.31	0.33	0.68	0.72
" " 11:45 A.M. after shaking 3 hours	0.35	0.46	1.01	1.24
" 30, 8:30 A.M.	0.42	0.46	1.08	1.56

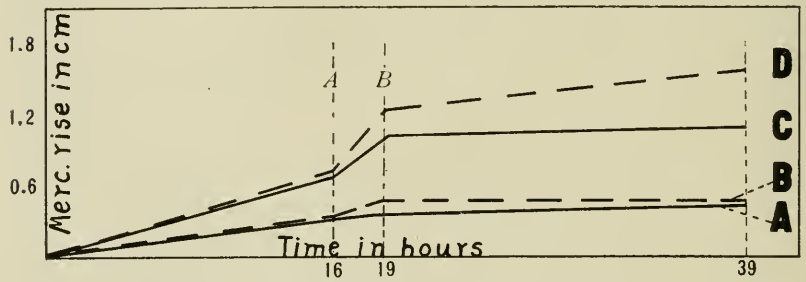

FIG. 6.—Oxidation of pyrogallol by precipitated oxidases from healthy and diseased bark, with and without gelatine, shaken only during period from *A* to *B:* A, precipitate from healthy bark without gelatine; B, precipitate from healthy bark with gelatine; C, precipitate from diseased bark without gelatine; D, precipitate from diseased bark with gelatine.

precipitates whose air dry weights, determined by the use of tared filters, were as follows:

	From extract of healthy bark	From extract of diseased bark
Fraction 1	0.0099 gm.	0.0532 gm.
Fraction 2	0.0080	0.0164
Total	0.0179	0.0696

The greater amount of precipitate from diseased bark may or may not be directly connected with its greater oxidase activity.

Further study is necessary to show the facts. A test of these precipitates with pyrocatechin showed that while the 2 fractions from healthy bark are about equal in oxidizing power the first fraction from diseased bark is 11 times as active as the second (fig. 7).

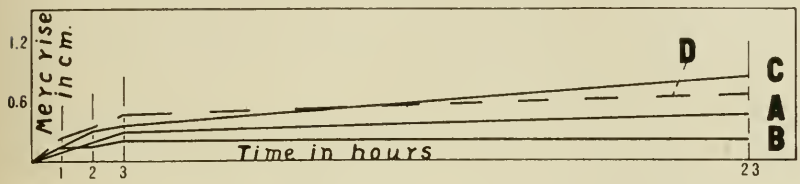

FIG. 7.—Oxidation of pyrocatechin by precipitated oxidases from healthy bark, without gelatine: A, fraction 1; B, fraction 2; C, fractions 1 and 2 tested together; D, sum of fractions 1 and 2 tested separately.

Other precipitates were prepared using 25 cc. of alcohol for the first fraction and 100 cc. more for the second. The oxidase activity of these, tested separately and combined, with and without gelatine, is shown in table XIX.

TABLE XIX

OXIDASE ACTIVITY OF FIRST AND SECOND FRACTIONS FROM BARK EXTRACT TESTED SEPARATELY AND COMBINED; TEMPERATURE 29.5–30.0° C.

BARK EXTRACT	WITHOUT GELATINE		WITH GELATINE	
	Sum of fractions 1 and 2 tested separately	Fractions 1 and 2 combined	Sum of fractions 1 and 2 tested separately	Fractions 1 and 2 combined
Healthy, after 23 hours....	0.65	0.84	0.76	0.84
Diseased, " 38 "	1.82	2.00	2.64	3.06

The mechanism by which gelatine increases the oxidase activity is not clear. It is evidently not through buffer action, as shown by its lack of effect on the hydrogen ion concentration (table XVII, figs. 8, 9, 10). Special tests showed that there was no hydrolysis of the gelatine to amino acids, in either healthy or diseased bark, which would increase its buffer effect. If gelatine is effective through its action as a protective colloid, its effect in this direction must be very complex, as shown by its difference in effect on bark mixtures and precipitated oxidases.

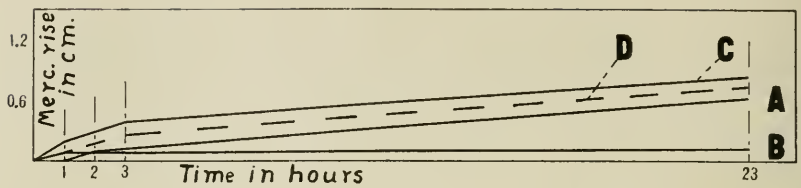

FIG. 8.—Oxidation of pyrocatechin by first and second fractions of healthy bark, with gelatine (for explanation of lettering see legend for fig. 7).

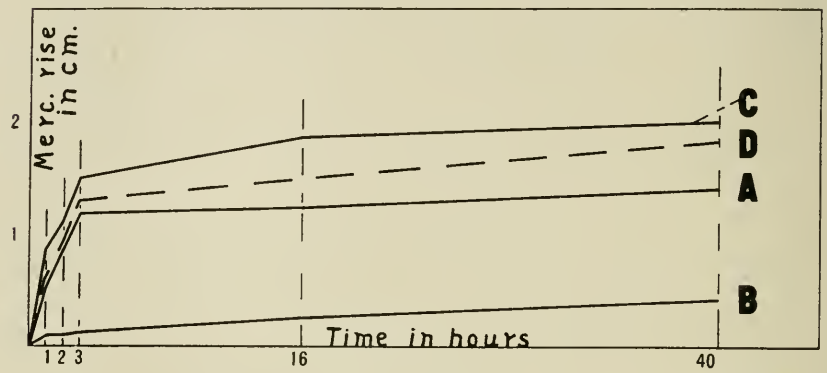

FIG. 9.—Oxidation of pyrocatechin by first and second fractions from diseased bark, without gelatine (for explanation of lettering see legend for fig. 7); points of plotting marked by vertical broken lines.

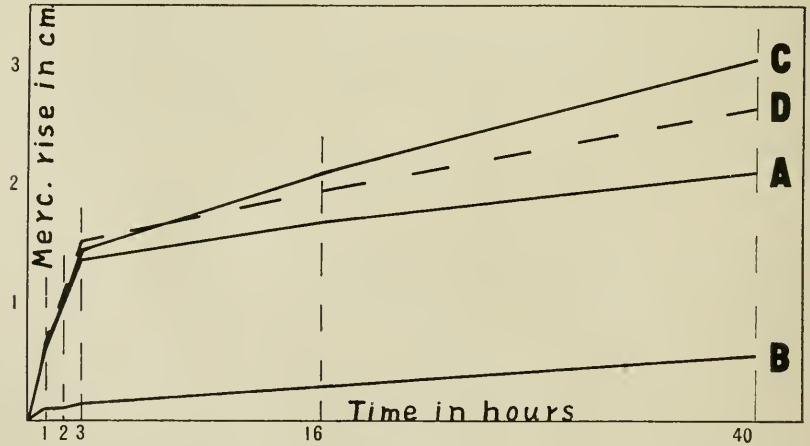

FIG. 10.—Oxidation of pyrocatechin by first and second fractions from diseased bark, with gelatine (for explanation of lettering see legend for fig. 7); points of plotting as in fig. 9.

The difference in the effect of gelatine and of gum arabic on oxidation by healthy bark may depend on differences in the colloidal solutions they form. An artificial oxidase prepared by DONY-HENAULT (18) from manganese formate, sodium bicarbonate, and gum arabic could be destroyed by heat; but one prepared by TRILLAT (33) from albumin and manganese could not be so destroyed. BAYLISS (6, p. 585) thinks the difference here "clearly depends on the nature of the emulsion colloid in association with the metal." On the other hand, what little increase in oxidation gum arabic produces may be due to an oxidase naturally present in it (BOURQUELOT 7), although an experiment designed to test this question gave negative results. One per cent gum arabic plus 1 per cent pyrogallol, and pyrogallol alone, were placed in separate oxidase tubes and shaken twice during each 24 hours. At the end of 3 days the mercury rise was 0.32 cm. in the first case and 0.20 cm. in the second, a difference almost within the limits of error in reading the manometers.

The data given in table XIX show that when the precipitate is collected in 2 fractions, these fractions have a greater oxidase activity if combined than if used separately. This condition seems to be about the same as that described by BACH and CHODAT (4) for *Lactarius vellereus*. They found that by the fractional precipitation of an aqueous solution of the oxidase of this fungus, by alcohol, 2 fractions could be obtained possessing markedly different properties. The first of these was almost insoluble in 40 per cent alcohol and had the properties of a weak oxidase; the second was soluble in 40 per cent alcohol but insoluble in pure alcohol and had no oxidizing powers. This fraction, however, was found to impart greater activity to hydrogen peroxide as an oxidizing agent; it was also found to increase markedly the oxidizing powers of the first fraction. The chief difference between this situation and that found in the work with apple bark is that in the latter case the first fraction has more than a weak oxidase activity, while the second, possibly because of incomplete separation of the fractions, is not entirely without it. No tests have been made of the behavior of the second fraction toward hydrogen peroxide.

OXIDASE ACTIVITY OF THE FUNGUS IN PURE CULTURE.—A fungus powder was prepared according to the method employed by REED (29) from mats of *Nummularia* mycelium grown in the potato extract medium described by DUGGAR (19). A test with 3 Bunzell tubes using 0.1 gm. of fungus powder, 4 cc. of 1 per cent pyrogallol, and 1 cc. of water gave after 4 days an average mercury rise of 2.35 cm. Quantitative tests on the medium in which the fungus had grown showed "oxidase" present there also. From these results it appears probable that the greater oxidase activity of diseased bark is due to a summation of the oxidase activity of normal bark and of the canker fungus itself. This may also account for the difference in behavior of the oxidases of the two.

The general conclusion to be drawn from the preceding data is that diseased bark has greater oxidase activity than healthy bark, probably because of lower acidity and greater degree of dispersion of the oxidizing agent, and because of an actually greater oxidase content. The lower tannin content of diseased bark (see macrochemical work) may also be a contributing factor, since tannins are known to cause inhibition of oxidase action. This factor is probably eliminated when precipitated oxidases are used.

In reference to the Bunzell apparatus it may be said that while it gives valuable comparative measurements of oxidase activity, those using it must realize its limitations. Conditions within it are artificial; with reference to hydrogen ion concentration, and probably other inhibiting factors, they are unstable and continually moving toward an equilibrium which, so far as we know, does not coincide with the equilibrium obtaining in the plant.

Catalase

Determinations of catalase activity (table XX) were made on 12 samples of bark, of which nos. 9 and 10 form a set from one tree and nos. 13 to 20 a set from another tree. Nos. 3 and 4 each came from different trees and are the ones used for most of the oxidase work reported in this paper. They were about 1 year old when tested for catalase. The other samples were freshly prepared for this work in December 1917 and January 1918. The limbs from which they came were carefully cleaned to remove lichens,

Pleurococcus, etc., since microchemical work had shown that such growths have a high catalase activity. The bark was then shaved off, ground in a meat chopper, and allowed to dry on filter paper at room temperature. In the case of samples 9, 10, 14, 16, 18, and 20, calcium carbonate was added during the grinding process at the rate of 0.5 gm. to each 10 gm. of unground bark, to prevent destruction of catalase by the acids of the bark (2) or of the hydrogen peroxide used. The dried bark was finally ground to a powder and only that part used which passed through an 80-mesh sieve.

TABLE XX

CATALASE ACTIVITY OF APPLE BARK

SAMPLE NUMBER	DESCRIPTION OF SAMPLE	TEMPERATURE	POSITIVE PRESSURE IN CM.	
			After 5 min.	After 10 min.
3	Healthy, from sound limb, no carbonate	23.5	0.10	0.10
4	Diseased, no carbonate	.....	0.55	0.95
9	Healthy, from sound limb, plus carbonate	21.0	0.83	1.26
10	Diseased, plus carbonate	.....	5.49	8.59
13	Healthy, from sound limb, no carbonate	21.0	0.52	0.70
14	Healthy, from sound limb, plus carbonate	.....	1.83	3.02
15	Healthy (?) 5 cm. from canker, no carbonate	22.0	0.54	0.65
16	Healthy (?) 5 cm. from canker, plus carbonate	.....	0.73	1.01
17	Diseased, no carbonate	20.5	0.55	0.81
18	Diseased, plus carbonate	.....	1.47	2.74
19	Dead, no carbonate	22.5	5.00	7.37
20	Dead, plus carbonate	.....	7.01	12.17

Tests were made at room temperature by means of the simplified Bunzell apparatus, using 0.03 or 0.10 gm. of bark powder, 1 cc. of water, and 4 cc. of 25 per cent hydrogen peroxide. After the experiment was set up the apparatus was allowed to stand for half an hour, when the manometers were closed and the solutions mixed. The apparatus was shaken for 10 seconds at the end of each minute and readings taken after 5 and 10 minutes. All tests were made in duplicate or quadruplicate, a water blank being included for temperature corrections as in the oxidase work.

A test for catalase was run also on the fungus powder previously mentioned, using 0.03 gm. in each tube and calculating the results to the basis of 0.10 gm. The average mercury rise (positive pressure) produced in 3 tubes was 1.65 cm. in 5 minutes and 2.57 cm. in 10 minutes, or, calculated to the basis of 0.10 gm., 5.49 cm. in 5 minutes, and 8.55 cm. in 10 minutes. It is worthy of note that a powder prepared from *Nummularia* mycelium grown in Raulin's solution, which is acid to litmus, showed no catalase activity. Experiments with different amounts of material showed that the positive pressure varies directly with the amount of material used. It was deemed legitimate, therefore, to calculate all results to the basis of 0.10 gm. of bark powder, and the figures for final tabulation were so calculated.

The results for samples 14, 16, 18, all from the same tree, show that diseased bark (sample 18) had more than twice the catalase activity of seemingly healthy bark 5 cm. away from the canker (sample 16), but only nine-tenths of that of bark from a sound unaffected limb on the same tree (sample 14). Dead cankered bark from this tree (sample 20) had 4 times the catalase activity of healthy bark, 12 times that of seemingly healthy bark next the canker, and nearly 5 times that of diseased bark. In the case of samples 9 (healthy) and 10 (diseased), the results are reversed, since the diseased had a catalase activity nearly 7 times greater than that of the healthy bark. The reason for the discrepancy between these two sets is not clear. The high catalase activity of sample no. 10 can hardly have been due to the presence of lichens, etc., or of an admixture of really dead bark, for precautions were taken when the samples were removed to avoid these sources of error. From the present data the only conclusion that can be drawn is that diseased bark from different trees varies considerably in its catalase activity, and that in general the more completely the bark is destroyed by the fungus the greater is its catalase activity. This condition is probably to be explained by the presence in the diseased bark of considerable amounts of mycelium which, as shown, produces a catalase of its own.

The seemingly healthy bark near the canker when compared with sound and with diseased bark appears to form an exception

in the series. Its catalase activity is less than that of either of the others and seems to be less affected by tissue acids when no carbonate is added. It is possible that near the canker the host's catalase is injured by materials from the fungus, even in advance of actual invasion by the hyphae. The fungus catalase may not appear here at all, but only later in the diseased bark, and in increasing amounts as the amount of mycelium increases.

The oxidase activity of samples 13, 15, 17, 19, together with the catalase activity of samples 14, 16, 18, 20, identical with them except for the addition of carbonate, are given in table XXI.

TABLE XXI

CATALASE ACTIVITY OF APPLE BARK

DESCRIPTION OF SAMPLE	MANOMETER READINGS EXPRESSED IN CM. OF MERCURY USING 0.1 GM. OF BARK POWDER	
	Catalase	Oxidase
Healthy	3.02	1.16
Healthy (?) 5 cm. from canker	1.01	1.47
Diseased	2.50	1.95
Dead	12.17	0.88

It will be seen that there is a gradual increase in oxidase activity from healthy to diseased bark, but a marked decrease in the case of dead bark. The catalase is considerably lower in apparently healthy bark near a canker than in the bark of an unaffected limb, but very much higher in the bark killed by the fungus than in bark from a healthy limb.

Microchemical analysis

Tests for oxidase, peroxidase, and catalase were made on fresh bark, all others on bark preserved in 50 per cent alcohol. The results are given in table XXII.

In making the tests for oxidase (direct action) and peroxidase (indirect action), the brownish purple color due to oxidation of benzidine was found most marked at first, in both healthy and diseased bark, in a zone 2 or 3 cells wide just inside the cork and in the pith rays. Later it came to about the same intensity over

the whole section. Catalase, judging by evolution of gas when H_2O_2 was added, was evenly distributed in all the tissues. Tests with $FeCl_3$ on sections of bark successively farther and farther

TABLE XXII

RESULTS OF MICROCHEMICAL TESTS ON HEALTHY AND DISEASED BARK

SUBSTANCE	REAGENT	REACTION	
		Healthy	Diseased
Cellulose	IKI and 75 per cent H_2SO_4	++	+
Pectin	Ruthenium red	+	+
Lignin	Phloroglucin and HCl	+ in bast	+ in bast
Tannin	Ferric chloride	++	+
Nitrates	Diphenylamine in 75 per cent H_2SO_4	.....	.....
Fats	Sudan III	+ Especially in parenchyma next to cork	+ Same as for healthy bark
Calcium (crystals)	50 per cent acetic acid	++ Crystals soluble	++ Crystals soluble
	50 per cent H_2SO_4	++ $CaSO_4$ formed	++ $CaSO_4$ formed
Calcium oxalate (crystals)	Oxalic acid	++ Crystals not changed	++ Crystals not changed
Direct reducing sugars	Flückigers reagent	+	++
Starch	IKI	++	+
Cyanogenic glucoside, probably amygdalin	Picric acid and Na_2CO_3	+	.....
	Berlin blue reaction	+	.....
Oxidase (direct action)	1 per cent benzidine in 50 per cent alcohol	+	+
Peroxidase (indirect action)	1 per cent benzidine and H_2O_2	+	+
Catalase	H_2O_2	+	+

distant from the badly browned region showed steadily increasing amounts of tannin. Pectin seemed to be present in about equal amounts in both healthy and diseased tissues.

Macrochemical analysis

Six samples were analyzed. The analytical methods used for 4 of them are based on those employed by KOCH for the quantitative study of animal and plant tissues (**21**, pp. 199–207). The difference in material required minor variations from these methods, but it is not thought necessary to describe them here. The other

2 were analyzed according to a method devised by KRAYBILL (unpublished work) in a study of the chemical composition of tomato plants. Material for 4 of the samples, healthy 1 and 2 and diseased 1 and 2, was taken from 8–10 cm. apple limbs cut in January at the Missouri State Fruit Experiment Station, and shipped from there by express. As soon as these samples arrived they were prepared as follows: bark designated as "healthy" was removed from sound limbs with a box scraper and cut into pieces half an inch square; about 150 gm. were then weighed quickly on a torsion balance to hundredths of a gram and put into enough redistilled alcohol (95 per cent) to give an alcohol concentration of approximately 85 per cent. The bottles containing the samples were then set into a steam bath until the alcohol came to a boil, then on top for 1 hour longer, to inactivate the enzymes.

Bark designated as "diseased" was taken from 8–10 cm. limbs showing well developed but not old cankers, usually about 45 cm. long. A strip of moist browned bark 2–3 cm. wide around the outside of the canker was removed with the box scraper, cut up, weighed, and preserved as described. This material usually contained small portions of the seemingly healthy bark outside of the canker, but never any part of the black dead material that often covers the central part of the cankered areas. Healthy samples 3 and 4 were taken from a 7 cm. limb cut in April when the bark peeled easily, to avoid removing small shavings of wood along with the bark, as was inadvertently done in the case of healthy samples 1 and 2 (see discussion of table XXV). Healthy samples 3 and 4 were not extracted with hot alcohol and ether as in the method described by HARVEY (21); instead the alcohol for preserving was filtered into a 1000 cc. flask and made up to volume. One-twentieth aliquots were then pipetted off into small beakers, evaporated to a syrup, and used later for dry weight and other estimations. The partly extracted bark was dried as described for the other samples, weighed, ground, allowed to come to air dry condition, and one-twentieth aliquots weighed out as before. This method of handling the material is much shorter than the KOCH method and is very satisfactory if one is not interested in the distribution of substances in the various fractions.

DRY WEIGHT.—One-tenth or one-twentieth aliquots, in tared crucibles or beakers, were brought to constant weight in a vacuum desiccator after intermittent drying for various lengths of time at about 100° C.

NITROGEN.—Estimations were made by the Kjeldahl-Gunning method, modified to include the nitrogen of nitrates. For healthy samples 1 and 2 and diseased samples 1 and 2 estimations were made separately on fractions 2 and 3; no nitrogen was found in fraction 2. Estimations for healthy samples 3 and 4 were made on one-twentieth of the alcohol extract combined with one-twentieth of the partly extracted bark.

CARBOHYDRATES.—Healthy samples 1 and 2, diseased samples 1 and 2: in the case of fraction 2, direct reducing sugars, and reducing sugars after mild hydrolysis, were estimated by the Bertrand volumetric method and calculated as dextrose by use of the MUNSON and WALKER tables (34). The more important details of manipulation, including precipitation of non-sugars, are given by CULPEPPER, FOSTER, and CALDWELL (16). The polysaccharides in fraction 3 were estimated as dextrose, but after 2.5 instead of 5 hours' hydrolysis (16).

Healthy samples 3 and 4: one-twentieth of the air dry, partly extracted bark was further extracted on a filter with about 200 cc. of water at 40° C., the filtrate being collected in a beaker containing one-twentieth of the alcohol extract. Estimation of sugars and polysaccharides in the combined extracts were then made as already described. The results of the analysis are given in tables XXIII and XXIV and summarized in table XXV.

The most important differences shown in the tables, as between healthy samples 1 and 2 and diseased samples 1 and 2, are as follows: diseased tissue contains 3.23 per cent more dry matter than healthy, although here much depends on the manner in which the sample is taken; on the basis of dry weight, fraction 1 is larger in the diseased by 4.56 per cent (nearly doubled), indicating a synthesis of lipoids by the fungus; fraction 3, the alcohol-water-insoluble residue, is larger by 1.83 per cent, while fraction 2, containing the alcohol-water-soluble substances, is smaller by 6.27 per cent. These results are strikingly similar to those found by

CULPEPPER, FOSTER, and CALDWELL (16), working with black rot of apples, caused by *Sphaeropsis malorum*. The increase in total

TABLE XXIII

RESULTS OF ANALYSIS OF HEALTHY BARK

Material	Percentage wet weight	Percentage wet weight	Percentage dry weight	Percentage dry weight
	Sample 1	Sample 2	Sample 1	Sample 2
Total solids	51.55	51.50	.....	.....
" " F_1	2.56	2.59	4.97	5.04
" " F_2	14.84	13.26	28.79	25.85
" " F_3	34.14	35.45	66.21	69.08
Total nitrogen	0.23	0.23	0.45	0.46
Direct reducing sugars	1.58	1.60	3.06	3.13
Reducing sugars after mild hydrolysis	0.55	0.54	1.07	1.05
Reducing sugars after strong hydrolysis F_1 and F_2	1.07	0.22	2.08	1.71
Reducing sugars after strong hydrolysis F_3	7.40	7.56	14.35	14.74
Reducing sugars after strong hydrolysis, total	8.47	7.78	16.43	16.45
	Sample 3	Sample 4	Sample 3	Sample 4
Total solids	46.13	46.24	.....	.....
Total nitrogen	0.217	0.231	0.46	0.50
Direct reducing sugars	0.915	0.949	1.96	2.05
Reducing sugars after mild hydrolysis	0.634	0.662	1.72	1.45
Reducing sugars after strong hydrolysis	7.524	7.189	16.20	16.55

TABLE XXIV

RESULTS OF ANALYSIS OF DISEASED BARK

Material	Sample 1 Percentage wet weight	Sample 2 Percentage wet weight	Sample 1 Percentage dry weight	Sample 2 Percentage dry weight
Total solids	54.29	55.12	.....	.....
" " F_1	4.69	5.77	8.64	10.47
" " F_2	11.52	11.52	21.21	20.89
" " F_3	38.07	37.94	70.16	68.80
Total nitrogen	0.45	0.45	0.83	0.81
Direct reducing sugars	1.42	1.56	2.62	2.83
Reducing sugars after mild hydrolysis	0.66	0.59	1.22	1.07
Reducing sugars after strong hydrolysis F_1 and F_2	0.13	0.38	0.23	0.70
Reducing sugars after strong hydrolysis F_3	8.80	8.84	16.21	16.04
Reducing sugars after strong hydrolysis, total	8.93	9.22	16.44	16.74

nitrogen in diseased bark may be due to fixation by the fungus or to a withdrawal of nitrogen from the surrounding tissue. Further data are necessary before a conclusion can be reached. CULPEPPER, FOSTER, and CALDWELL found protein-nitrogen content of fraction 2 for diseased apples larger than for normal ones, but the total nitrogen for the whole tissue smaller for the former than for the latter.

TABLE XXV

SUMMARY

MATERIAL	AVERAGE PERCENTAGE WET WEIGHT			AVERAGE PERCENTAGE DRY WEIGHT		
	Healthy 1 and 2	Healthy 3 and 4	Diseased 1 and 2	Healthy 1 and 2	Healthy 3 and 4	Diseased 1 and 2
Total solids	51.53	46.19	54.70	.....	.....	.....
" " F_1	2.58	.....	5.23	5.00	.....	9.56
" " F_2	14.05	.....	11.52	27.32	.....	21.05
" " F_3	34.00	.....	37.57	67.65	.....	69.48
Total nitrogen	0.23	0.24	0.45	0.46	0.48	0.82
Direct reducing sugars	1.59	0.93	1.49	3.00	2.00	2.73
Direct reducing sugars after mild hydrolysis	0.55	0.65	0.62	1.06	1.59	1.15
Direct reducing sugars after strong hydrolysis	8.12	7.35	9.08	16.44	16.38	16.59

Results with healthy samples 3 and 4 furnish little of additional interest. They show, however, that as far as total nitrogen and starch are concerned, the small amount of wood in the other 2 healthy samples had no effect on the results. The difference in the case of dry weight and reducing sugars before and after hydrolysis is probably due to the fact that samples 3 and 4 were taken from a limb cut early in the growing season, while samples 1 and 2 were taken from limbs cut in the dead of winter.

Estimation of tannin

The method used was that of LÖWENTHAL, as modified by PROCTOR (**34**, p. 150). Material for analysis was taken from 8–12 cm. Ben Davis limbs cut in November, December, and January. The bark was cut off as already described, ground in a meat grinder, and transferred to a glass moist chamber at once. About 10 gm. were then weighed out and set to boil in 400 cc. of

water as required by the LÖWENTHAL method; at the same time duplicate samples were taken for moisture determination. Whatever may have been the errors introduced by this method, the agreement between duplicates taken for moisture determination was very close in most cases, as is shown in table XXVI.

TABLE XXVI

PERCENTAGE OF DRY MATTER IN DUPLICATE SAMPLES OF VARIOUS LOTS OF BARK ANALYZED

Sample	Duplicate 1	Duplicate 2	Average
Healthy 1	47.89	47.65	47.77
" 2	45.96	45.76	45.86
" 3	46.95	46.86	46.91
Diseased 4	50.44	49.53	49.99
" 5	49.98	49.41	49.69
" 6	49.86	49.73	49.80
Dead 7	56.47	.....	.....
" 8	64.85	64.74	64.79
" 9	76.34	75.83	76.10

The results of the analysis of 9 different samples of bark are shown in table XXVII.

TABLE XXVII

PERCENTAGE OF TANNIN IN HEALTHY AND DISEASED APPLE BARK

Description of sample	Tannin (percentage dry weight)
Healthy	
1	5.16
2. 8–10 cm. from canker	3.64
3. From same tree as 6 and 9	3.38
Average	4.06
Diseased	
4.	2.49
5. From same limb as 2	3.56
6.	2.93
Average	2.99
Dead (from surface of canker)	
7. From same limb as 1	0.25
8.	1.14
9.	1.51
Average	0.97

The LÖWENTHAL method probably determines merely the easily water-soluble tannins, but fails to reach those tied up with

the suberin. If suberin is for any reason more abundant in the diseased bark, an error would thus be introduced which might invalidate any comparisons based on the results obtained. Subject to this possible correction the results shown in table XXVII confirm those obtained in the microchemical analysis; that is, they show a progressive decrease in tannin as the bark is more and more affected by the disease. Healthy bark was found to contain on the average 4.06 per cent of tannin, diseased 2.99, and dead 0.97. If sample 1 healthy, which gave a high figure, and sample 7 dead, which gave a low figure, be eliminated, the averages become healthy 3.51, diseased 2.99, dead 1.33. The figures for samples 3, 6, and 9, all from the same tree, are healthy 3.38, diseased 2.93, dead 1.51. There is undoubtedly a difference between bark from a sound limb and seemingly healthy bark from a limb that is badly cankered. The latter is usually slightly browned throughout when first cut off and rapidly becomes reddish brown on exposure to air. Really healthy bark under such conditions shows only a slight browning.

Whatever the results with apple bark may mean, they are not in agreement with the statement made by KERR (see COOK and WILSON, **15**, p. 26, footnote) that because of the greater stability of tannin and the disappearance of other constituents "all decayed wood and bark give higher tannin contents, no matter what causes the decay." If confirmed by further analyses they would indicate a different relation between host and parasite with reference to tannin in the case of blister canker than obtained in any of the cases studied by KERR. Leaching of tannin may account for the low percentage found in dead apple bark, as suggested by the chemist of the Chestnut Tree Blight Commission (**15**, p. 6) for old cankers of chestnut blight, but can hardly be responsible for the condition found in diseased bark.

Summary

1. Measurements with the simplified Bunzell apparatus show that apple bark attacked by *Nummularia discreta* causes about twice as much oxidation of pyrogallol, pyrocatechin, guaiacol, and benzidine as does healthy bark.

2. The gradual slowing down of oxidation in the Bunzell apparatus is shown to be due, in part at least, to increasing hydrogen ion concentration, brought about by the oxidation process itself. The equilibrium reached in the oxidase apparatus seems to be a false one, which can be disturbed by the addition of either fresh oxidase reagent or plant material. When tested by the formula for a unimolecular reaction, the oxidase reaction gives values for k, which indicate clearly a linear relationship between time and amount of change and suggest that the oxidase is a catalytic agent.

3. The hydrogen ion concentration of diseased bark ($P_H = 5.61$) is definitely less than that of healthy bark ($P_H = 5.15$). Work with buffer solutions shows that this difference is not great enough to account for all of the difference in the oxidase activity of the two kinds of material. When mixtures of the two are brought to the same hydrogen ion concentration by means of buffer solutions, diseased bark still shows greater oxidase activity.

4. The temperature and duration of drying have an effect on the acidity and the oxidase activity of both healthy and diseased bark.

5. Eight-tenths per cent gelatine increases the oxidase activity of both kinds of bark. This may be due to the action of gelatine as a protective colloid which prevents precipitation of the "oxidase." It is not due to buffer action.

6. The concentration of hydrogen ion necessary or complete inhibition of oxidase activity of healthy bark lies between 3.55 and 3.80×10^{-4}; for that of diseased bark between 3.55 and 4.27×10^{-4}.

7. Oxidation in the apparatus comes to an end only after several days instead of after a few hours, as stated by BUNZELL.

8. When the "oxidase" is precipitated in 2 fractions, the first has greater oxidizing power than the second, and the 2 combined have slightly greater oxidizing power than when tested separately.

9. Catalase determinations gave the following results: healthy 3.02 (cm. positive pressure), seemingly healthy 5 cm. from the canker 1.01, diseased 2.74, dead 12.17; results from oxidase determinations for the same stages were 1.16, 1.47, 1.95, 1.85 (cm. negative pressure). These results show some discrepancies, but justify the general statement that the more severely the bark

is attacked by the fungus the greater is its catalase activity, and that catalase activity in part is in indirect ratio to oxidase activity.

10. Microchemical tests indicate, for diseased bark, a partial disintegration of cellulose, a disappearance of cyanogenic glucoside, and a lower content of starch, calcium oxalate, and tannins.

11. Macrochemical analyses show that diseased bark has a higher percentage of dry matter, lipoids, alcohol-water-insoluble residue, and total nitrogen, but a lower percentage of alcohol-water-soluble material than healthy bark. The percentage of carbohydrates in both tissues seems to be about the same. Differences in tannin content are definite but not large. Sound healthy bark contains more than diseased bark and diseased bark more than dead bark from the surface of the canker.

12. The greater oxidase activity of diseased bark is probably due to the combined activity of the oxidases of fungus and host, lower acidity, and possibly to a greater degree of dispersion of the oxidizing agent. The lower tannin content of diseased bark may also be a contributing factor.

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U.S. DEPARTMENT OF AGRICULTURE
WASHINGTON, D.C.

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MORPHOLOGY OF THE GENUS ACTINOMYCES. II

CHARLES DRECHSLER

(WITH PLATES II–IX)

Taxonomy of species

The obscurity that has surrounded the morphology of *Actinomyces*, besides involving the genus in improbable speculations concerning its phylogenetic relationship to the bacteria, has brought about also a most unfortunate condition in the taxonomy of the many described species. Most of the work on the genus has been done by investigators with bacteriological inclinations, and even where this has not been true, the prevalent view of the nature of these minute plants has lead to the adoption of methods scarcely applicable to mycological research. A discussion of characteristics like the occurrence of endospores, flagella, capsules, sheaths, and involution forms, cannot be regarded as constituting a morphological treatment more satisfactory for species of *Actinomyces* than for species of *Mucor* or *Boletus*.

The dependence of certain biochemical processes, particularly chromogenesis, on definite conditions of nutrition, and the conspicuous differences resulting from comparatively slight changes in the substratum, have long been noted by students of *Actinomyces*, yet descriptions of new species have continued to appear, based largely and often quite exclusively, on these variable activities. Very frequently writers have not compared their organisms with others reported by previous investigators; and in recent years there has been a tendency to disregard altogether the taxonomic contributions of the preceding decades. Moreover, while identities have frequently not been recognized where they existed, in other cases organisms have been supposed to be identical on extremely slight evidence. One of these cases that has led to an unusual measure of confusion is that of GASPERINI'S *Actinomyces chromogenus*. This species was identified by both GASPERINI and ROSSI-DORIA (19) with an organism isolated from the air by the

latter and designated as *Streptothrix nigra*. In culture it was characterized by a dark brown or black discoloration of certain kinds of substrata, a reaction easily obtained on potato agar, for example, and ascribed by LEHMAN and SANO (13) to the production of tyrosinase. Until recently it has been the custom among writers to refer nearly every member of the genus showing a tyrosinase reaction to *Actinomyces chromogenus*, LUTMAN and CUNNINGHAM (14) going so far as to identify this species with the potato scab organism. This practice, which would unite forms so different in appearance and method of development as, for example, *Actinomyces* I and III, regardless of pronounced differences in size and in dextrorse or sinistrorse condition, is not defensible on morphological grounds. KRAINSKY resolved the "chromogenus" complex into 4 species; while WAKSMAN and CURTIS increased the number of derivatives to 8. Of the 17 morphologically distinct saprophytic species figured in this paper, 11 exhibit a tyrosinase reaction; and these represent less than one-fifth of the number of similarly active species which the writer had occasion to examine.

The genus awaits the attention of an investigator in a position to make a comprehensive study involving at least the larger proportion of species existing within wide geographical ranges. The summaries given later, of the more important facts about each species selected for morphological treatment, are not to be regarded as descriptions intended for taxonomic purposes.

ACTINOMYCES I

CULTURAL CHARACTERS.—On glucose agar (0.5 g. peptone, 10.0 g. glucose, 20.0 g. agar, 1000 cc. tap water) nutritive mycelium of individuals smooth, opalescent, more or less confluent; sporulation moderately slow and commencing as a light creamy zone near the periphery; no diffusible stain. On potato agar (decoction of 200 g. peeled potatoes, 2.0 g. glucose, 20.0 g. agar, 1000 cc. water) nutritive mycelium light olivaceous; sporulation moderately abundant, the raised areas where the yellowish gray fructifications are to appear being previously distinguishable by a deep brownish green coloration; guttation never copious, often absent; tyrosinase reaction moderate, but distinct.

Morphology.—The development of the erect sporogenous hyphae of this species is strictly successive, and may be followed in the branch *d* in fig. 2, the younger of any 2 hyphae being distinguishable by its attenuated attachment. The partly disrupted chain of spores *d1* here represents the original prolongation of branch *d;* the chain *d2* represents a secondary branch, the spores here being mature but still retaining their spiral disposition without showing indications of disruption; while *d3* represents a tertiary branch, in which septation has not commenced. A similar sequence is illustrated in the succession of derivatives *b1*, *b2*, *b3*, and *b4* from the branch *b*, as well as in the 5 elements *c1–c5* associated with the branch *c*.

A more complex system of fertile hyphae is shown in fig. 1, but the larger fructifications are probably 10 or even 15 times more extensive, and bear many thousands of spores. The species is characterized by close sinistrorse spirals, of 2–6 turns, and 3–4 μ in diameter, which during the later stages of maturation are relaxed, although indications of them may persist in the flexuous or sinuous course of the mature chains of spores. The mature spores are ellipsoidal, 1–2-nucleated, with a distinctly visible wall and a central vacuole of varying size. They measure 1.2–1.4 × 1.4–20 μ, and upon germinating produce 2–4 germ tubes, which early proliferate numerous branches, and show at intervals some dark staining granules.

Isolated 5 times from soil collected in Cambridge, Massachusetts.

ACTINOMYCES II

Cultural characters.—On glucose agar, growth moderate; nutritive mycelium colorless, early covered with a cretaceous or downy aërial mycelium; pigment absent. On potato agar, development of nutritive mycelium moderately rapid; aërial mycelium appearing in scattered areas, first white, later becoming slightly discolored; substratum stained yellow by a soluble pigment; tyrosinase reaction absent.

Morphology.—The most conspicuous feature of this species is the extraordinary thickness of the septa (0.3–0.35 μ) associated

with spore production, and their insertion at distensions in the sporogenous hyphae. A comparison of branch *c1* with the younger branch *c2* (fig. 5) corresponding to the conditions shown diagrammatically in figs. 8*b* and 8*c* respectively, shows that the growth in thickness of the hyphae takes place subsequent to the appearance of the septa. After the filament has attained its growth, the septa split along a median plane (figs. 5*a*, 5*c1*, 8*d*), and the 2 halves are drawn apart by the contraction of the delimited protoplasts. Further maturation occurs in the distribution of the deeply staining wall substance, in the strengthening of the peripheral wall, and by an enlargement of the latter, the elimination of the median, hourglass-like constriction of the spore, resulting finally in an approximately cylindrical structure measuring 0.7–0.9×0.8–1.1 μ.

The terminal and the basal spores of each chain retain a somewhat asymmetrical shape, owing to the absence of the massive septum at one of their ends. By an apparently abnormal development, metachromatic granules may be formed in the spores derived from some hyphae, resulting in a condition illustrated in the lowest spore in fig. 8*f*.

The axial filaments are represented by long prostrate hyphae, branching at irregular, long intervals. Septation is confined to the fertile branches. The sterile hyphal portions below the sporogenous terminations taper gradually toward their attenuated attachments. Development and sporogenesis near the axial terminations are successive, and involve the formation of close sinistrorse spirals of 1–5 turns, 3.5–6.0 μ in diameter.

Isolated 3 times from soil collected in Cambridge, Massachusetts.

ACTINOMYCES III

A. lavendulae Waksman and Curtis

CULTURAL CHARACTERS.—On glucose agar, nutritive mycelium slightly yellow on reverse side, central area completely covered with velvety aërial mycelium, first white but gradually assuming a beautiful lavender shade; no soluble stain. On potato agar, growth very profuse; mycelium abundant, changing from white to lavender; guttation moderate to profuse; tyrosinase reaction vigorous.

Identity with *A. lavendulae* established by comparison of cultural and morphological characteristics.

Morphology.—The mycelium consists of long prostrate axial filaments, branching rarely except at the end. Sporulation is usually initiated at the tip of the filament, and proceeds basipetally by the insertion and transformation of almost invisible septa, to the point of attachment of the first sporogenous branch (figs. 10, 11). The sporogenous branches are rarely crowded, although at the base of the sporogenous axial termination an opposite arrangement is not uncommon. Secondary branching occurs frequently; septa are entirely absent, except when associated with the progressive basipetal delimitation of spores.

The sporogenous hyphae terminate in dextrorse, moderately compact spirals of 4–12 turns, 2.0–3.8 μ in diameter. The spores are ellipsoidal, with nuclei not readily demonstrable. Metachromatic material occurs abundantly in many old hyphae (fig. 15).

Isolated 3 times from soil collected in Cambridge, Massachusetts.

ACTINOMYCES IV

Cultural characters.—On glucose agar, nutritive mycelium colorless; aërial mycelium moderately profuse, velvety in appearance, changing from white to smoky blue; no guttation. On potato agar, tyrosinase reaction vigorous; aërial mycelium first produced white, not subsequently much discolored, becoming matted to the substratum as a result of excessive guttation, and later completely overgrown by a loose growth of smoky blue secondary mycelium.

Morphology.—The sporogenous branches with dextrorse spirals of 2–12 open turns, 1.5–2.5 μ in diameter, are attached to the long axial filaments usually at wide intervals, in a loose racemose arrangement. Secondary branching, although rare, occurs occasionally, and is then associated with simultaneous sporulation (fig. 18). Development of the 1–2-nucleated spores, 0.7–0.8 $\times$ 0.9–1.1 μ, proceeds by the insertion of conspicuous septa, followed by their constriction and subsequent conversion to hyaline isthmuses (figs. 70*a–e*). Two germ tubes are usually produced, of a more or

less uniform diameter, and proliferating branches at relatively wide intervals.

Isolated twice from soil collected in Cambridge, Massachusetts.

ACTINOMYCES V

CULTURAL CHARACTERS.—On glucose agar, nutritive mycelium on reverse side slightly yellowish; the surface completely covered with a luxuriant velvety or cottony weft of pinkish-yellow aërial mycelium; guttation slight. On potato agar, nutritive mycelium chocolate-colored, firm lichenoid, crimped around margin; aërial mycelium, as on glucose agar, but less profuse; tyrosinase reaction vigorous.

MORPHOLOGY.—The fertile hyphae, which are attached to prostrate axial filaments at long intervals, are terminated by relatively close sinistrorse spirals of 4–12 turns 2.0–4.0 μ in diameter, developing spores (0.6–0.8×0.9–1.1 μ), like *Actinomyces* IV, by the insertion of conspicuous septa, followed by their constriction and conversion. A peculiar characteristic is found in the sterilization of the basal portion of the fertile hyphae, by an apparent abortion of its lower potential spores.

Isolated 3 times from soil collected in Cambridge, Massachusetts.

ACTINOMYCES VI

CULTURAL CHARACTERS.—On glucose agar, nutritive mycelium colorless, completely covered with a felty aërial mycelium, first white, later assuming a deep smoky tinge. On potato agar, nutritive mycelium excessively wrinkled, partially covered with a slightly discolored aërial mycelium; tyrosinase reaction vigorous.

MORPHOLOGY.—The species appears closely allied to *Actinomyces* V, differing from the latter chiefly in the absence of any evidence of sterilization, and in the shorter length of its sinistrorse sporogenous spirals, which consist of only 2–6 turns, 2.0–4.0 μ in diameter. The spores are uninucleated, measure 0.7–0.8×0.9–1.1 μ, and are developed by the insertion and transformation of conspicuous septa. Fertile hyphae are attached to the axial fila-

ments with considerably greater frequency, and secondary branching, characterized by the successive type of development, is common.

Isolated once from soil collected in Cambridge, Massachusetts.

ACTINOMYCES VII

CULTURAL CHARACTERS.—On glucose agar, nutritive mycelium colorless, early developing an aërial mycelium from the center outward, the latter changing from white to light gray with increasing age. On potato agar, nutritive mycelium luxuriant, developing rapidly; aërial mycelium represented by a slight cretaceous development toward the top of the slant; tyrosinase reaction vigorous.

MORPHOLOGY.—This species departs from the main trend of the 3 preceding forms in the relatively close arrangement of its branches on the axial filament, and in the elaboration of these branches by further ramifications in a typically successive order. Nearly spherical to ellipsoidal spores, 0.6–0.8×0.7–1.0 μ, are produced from moderately close sinistrorse spirals of 3–8 turns 2.0–3.0 μ in diameter, by the development represented in fig. 70*a–e*, but the septa are relatively thin, and occasionally fall below the limit of clear visibility.

Isolated twice from soil collected in Cambridge, Massachusetts.

ACTINOMYCES VIII

CULTURAL CHARACTERS.—On glucose agar, nutritive mycelium nearly colorless, secreting a diffusible yellow pigment; aërial mycelium moderately profuse, velvety, first white, later changing to a light bluish color. On potato agar, growth similar, but soluble pigment absent; no tyrosinase reaction.

MORPHOLOGY.—The fertile hyphae of this species may be attached to the axial filaments in a diffuse racemose arrangement (fig. 46), or crowded in a compact capitate system. The swellings in the axial filaments in figs. 43, 44, 45, and 46 at the bases of sporogenous branches indicate the mode of origin of the *Leptomitus*-like distensions shown in figs. 47 and 48.

The small, ellipsoidal, uninucleated spores, 0.5–0.6×0.6–0.8 μ, are formed from close, sinistrorse spirals of 2–10 turns 1.2–2.5 μ in diameter. Indications of septa can be seen only rarely. The mature spore chains upon collapsing cohere in irregular zoogloœa-like masses, a peculiarity of behavior dependent probably on a gelatinization of wall material. Upon germination, 1 or 2 tubes are produced, relatively thick and abundantly branching.

Isolated 6 times from soil collected in Cambridge, Massachusetts.

ACTINOMYCES IX

CULTURAL CHARACTERS.—On glucose agar, nutritive mycelium colorless, forming no soluble pigment; aërial mycelium at first white, becoming light smoky blue in the course of a few days. On potato agar, cultural characters similar, but growth more profuse, guttation moderate, discoloration of aërial mycelium more rapid; tyrosinase reaction absent.

MORPHOLOGY.—The most characteristic feature of this species is the greater thickness of the fertile hyphae below the second turn of the spiral. The latter are sinistrorse, usually with very close turns, varying in number from 1 to 16, and measuring 1.5–2.0 μ in diameter. They give rise to ellipsoidal, uninucleated spores, 0.5–0.7×0.6–1.0 μ, without the appearance of clearly visible septa. It seems highly probable that cross-walls nevertheless occur, since occasionally a median partition may be differentiated in the hyaline attentuated connections between two spores (fig. 51), suggesting a development similar to that indicated in fig. 70*a–e*.

Isolated once from soil collected in Cambridge, Massachusetts.

ACTINOMYCES X

Streptothrix alba Rossi-Doria; *Actinomyces griseus* Krainsky (?)

CULTURAL CHARACTERS.—On glucose agar, growth poor and not characteristic. On potato agar, growth excessively rapid, nutritive mycelium colorless; aërial mycelium firm, white, changing rapidly to a yellowish gray; secondary growth occurring in the formation of numerous successive rings of sporodochia, or in the development of cottony white masses of mycelium from below the thick

crust of old mature spores; tyrosinase reaction absent; substratum stained a faint greenish yellow in old cultures.

Morphology.—According to Waksman and Curtis, the aërial filaments of this species possess only a slight tendency to branch. The writer was led to a somewhat different conclusion, as the axial hyphae are usually found to proliferate fertile branches at moderately close intervals. Occasionally, as in fig. 58, indications of a successive sequence may be observed, but more frequently the development of the different elements of a ramifying system occurs without any recognizable interrelation. The short, cylindrical spores, 0.7×0.7–1.0 μ, are formed, as in *Actinomyces* XVI, by a septation of the fertile hyphae, followed by splitting of the partitions along a median plane, but the septa are usually less conspicuous, and often not clearly visible, and the fertile hyphae show no indication of a spiral condition.

A striking dimorphism characterizes the mycelium of this species, as well as that of a number of other forms observed by the writer. The deeper sterile aërial hyphae below the sporogenous layer typically are extremely minute, with a diameter frequently not exceeding 0.3 μ; their protoplasmic contents show little affinity for stains; and the contours of their walls are uniformly smooth. The more superficial hyphae, which usually attain a thickness of 1.0 μ, and are distinguishable by markedly irregular contours, contain dense deep staining protoplasm; and when septa are present, they are sometimes associated, as in *Actinomyces* XVII and XVIII, with spherical structures. The thicker filaments bear the sporogenous branches, and, in general, appear to constitute the expanded prolongations of the minute hyphae (fig. 59).

Isolated twice from soil collected in Cambridge, Massachusetts; once from tap water; very frequently from outdoor air; several times from gross cultures of dead leaves; 4 times from horse dung undergoing fermentation at 50–60° C.

Synonomy.—In his description of *Streptothrix alba*, Rossi-Doria records two characteristics that establish its identity beyond much danger of confusion: a conspicuous preponderance in number over any of its congeners on plates exposed to the air, and a tendency toward the formation of concentric rings more pronounced than that of any other species. Rossi-Doria

attributed this preponderance in the air to its omnivorous character, enabling it to develop on a large variety of substrata, "Questa *Streptothrix* cresce, si può dire, dappertutto, tanto su terreni di natura vegetale quanto su terreni di natura animale. E per ciò nonchè la grande sua produzione di spore, che essa si trova cosi diffusa nell'aria ed altrove. Pare che essa possa svilupparsi anche nel terreno." In spite of this fortunate and quite distinctive characterization, the specific term "albus" subsequently came to be used in a manner as miscellaneous as "chromogenus," being applied generally to any type with a light mycelium showing no tyrosinase reaction.

The same species was treated in the publication of WAKSMAN and CURTIS as *Actinomyces griseus* Krainsky. I have not been able to satisfy myself fully about the identity of KRAINSKY'S organism; nor would it seem possible to reach any definite conclusion without an examination of authentic material.

ACTINOMYCES XI

CULTURAL CHARACTERS.—On glucose agar, nutritive mycelium first colorless, becoming slightly reddened with increasing age; aërial mycelium first white, rapidly changing to a bluish violet. On potato agar, nutritive mycelium gradually becomes deep red by the slow accumulation of a slightly diffusible pigment; tyrosinase reaction absent.

MORPHOLOGY.—More or less erect fructifications are developed along the distal portions of long prostrate filaments. Branching is abundant and only occasionally shows indications of a successive sequence. The aërial hyphae in the dendroidic structures (figs. 64, 66) are often conspicuously vacuolate, especially in the inflated distensions from which a number of fertile branches arise. The latter terminate in sinistrorse spirals of 4–6 turns, 2.0–3.0 μ in diameter, from which, by the insertion of conspicuous septa and their subsequent transformation to hyaline isthmuses, spores 0.5–0.7 × 1.0–1.2 μ are produced.

Isolated once from soil collected in Cambridge, Massachusetts; identical with an organism isolated by Mr. H. J. CONN from soil collected near Geneva, New York.

ACTINOMYCES XII

A. aureus Waksman and Curtis

CULTURAL CHARACTERS.—On glucose agar, nutritive mycelium yellowish on reverse side; aërial mycelium changing from white to pale yellowish gray; soluble stain absent. On potato agar, nutritive mycelium darker on reverse side; aërial mycelium more profuse, forming a somewhat more deeply colored felty layer; tyrosinase reaction moderate. Identity with *Actinomyces aureus* established by comparison with authentic material of the latter.

MORPHOLOGY.—In this species long prostrate filaments terminate in more or less erect fructifications. Secondary branches are proliferated from the lateral elements, generally in successive sequence. A more or less pronounced cuneate thickening of the hyphae below the insertion of a branch is characteristic of the species. The ellipsoidal, uninucleated spores, $0.5–0.7 \times 0.8–1.2\ \mu$, are formed by the insertion of conspicuous septa in open, sinistrorse spirals of 2–7 turns, $3.0–4.0\ \mu$ in diameter.

Isolated twice from soil collected in Cambridge, Massachusetts.

ACTINOMYCES XIII

CULTURAL CHARACTERS.—On glucose agar, nutritive mycelium light orange-brown, the separate individuals fused into a massive pellicle with a depressed, crimped margin. On potato agar, nutritive mycelium dark chocolate-brown, wrinkled, lichenoid, secreting a diffusible red pigment; tyrosinase reaction absent. Aërial mycelium on both substrata loose, cottony; developing slowly, first white, later changing to a dull bluish tint.

MORPHOLOGY.—The aërial mycelium consists of extremely long filaments, which rarely show any evidence of branching (figs. 74 75), and toward their terminations follow an undulating or slightly spiral course. Sporulation occurs as the result of protoplasmic contractions without the appearance of visible septa, the chains of cylindrical spores, $0.4 \times 1.2–1.6\ \mu$, being held together for some time by the evacuated portions of hyphal wall, that seem to undergo no apparent constriction.

Isolated 3 times from soil collected in Cambridge, Massachusetts.

ACTINOMYCES XIV

CULTURAL CHARACTERS.—On glucose agar, nutritive mycelium usually colorless, but frequently becoming deep brown or black; aërial mycelium consisting of a dense velvety weft, first white, later changing to a creamy yellow. On potato agar, growth similar; tyrosinase reaction absent.

MORPHOLOGY.—This species is characterized by the production of extensive prostrate fructifications through the proliferation of numerous lateral branching processes from long axial filaments (figs. 76, 79, 81). A septum is occasionally present immediately above the attachment of a branch, but more frequently is absent. Secondary ramifications, resulting in more or less complex elements, take place without reference to the stage of sporogenesis in the proliferating branch. The ellipsoidal uninucleated spores, 0.5–0.7×0.8–1.2 μ, are derived from sinistrorse spiral hyphae of 1–8 turns, 2.0–4.0 μ in diameter, by the insertion and transformation of relatively thin septa, or without the appearance of demonstrable septa.

Isolated 4 times from soil collected in Cambridge, Massachusetts.

ACTINOMYCES XV

CULTURAL CHARACTERS.—On glucose or potato agar, nutritive mycelium opalescent; aërial mycelium first white, becoming only slightly discolored with age; tyrosinase reaction moderate.

MORPHOLOGY.—Microscopically this species closely resembles *Actinomyces* IV, differing from the latter chiefly in the abundant proliferation of branches of the second or of a higher order. The lateral elements thus formed follow the successive type of development (figs. 82, 83). The uninucleated spores, 0.7×0.9–1.0 μ, are formed from dextrorse spiral hyphae of 3–12 turns, 1.8–2.5 μ in diameter, by the constriction of conspicuous septa, and their transformation into hyaline isthmuses.

Isolated twice from soil collected in Manhattan, Kansas.

ACTINOMYCES XVI

CULTURAL CHARACTERS.—On glucose agar, growth very meager; never producing an aërial mycelium. On potato agar, develop-

ment rapid; nutritive mycelium dark brown or greenish brown; aërial mycelium profuse, changing from white to violet or pinkish gray; guttation profuse; tyrosinase reaction moderate.

Morphology.—In this species the characteristic development consists in the proliferation of a number of long branches in an irregular whorl from a long and somewhat thickened axial filament. Secondary branching is common, but usually more or less remote. Vacuoles associated with hyphal distensions are found in the axial filaments and in the main branches, and metachromatic granules occur abundantly in many of the older sterile hyphae (fig. 91). The long cylindrical spores, 0.6–0.7×1.0–2.0 μ, are formed by the septation of sporogenous hyphae that terminate in open, sinistrorse spirals of 2–3 turns, 4.0–5.5 μ in diameter, followed by the splitting of the septa along a median plane, and the separation of the two halves by a contraction of the delimited protoplasts. The progress of sporogenesis is usually basipetal, but not infrequently the first divisions may result in a number of segments of varying lengths, which by subsequent divisions are reduced to the magnitude of the ultimate spores.

Isolated once from soil collected in Cambridge, Massachusetts.

ACTINOMYCES XVII

A. scabies (Thaxter) Güssow (6)

Morphology.—The aërial mycelium of this species, which is one of the largest of dextrorse forms, consists of long prostrate filaments on which lateral branches are inserted at short intervals. Secondary branching is abundant and usually associated with a successive order of development (figs. 93*a1–a3*). The more or less cylindrical spores, 0.8–0.9×1.3–1.5 μ, are developed from dextrorse spiral hyphae of 3–14 turns, 2.0–3.5 μ in diameter, by the insertion of conspicuous septa and their subsequent splitting along a median plane. In many hyphae the septa before their division can be seen to occupy a transverse equatorial position in the peculiar spherical structures to which reference has been made elsewhere, and which here occupy slight but perceptible hyphal distensions (figs. 92, 93*h1*, 93*h2*, 101*cy*). Whenever the spherical structures

are absent, the fertile hyphae are uniformly isodiametric. It is not certain whether these structures appear in all sporogenous branches at some time preceding the contraction of the delimited protoplasts, or are more or less accidental in their occurrence. They also are found associated with septa in the sterile axial filaments, and here similarly occupy local hyphal distensions. After the individual spores have become separated, the connecting segments of evacuated hyphal wall contract slightly to form somewhat narrowed isthmuses, which persist until the mature spore chains are disrupted. In germinating, the spore usually produces 1 or 2 germ tubes.

The preparation from which figs. 92–101 were drawn was derived from one of 5 organisms communicated by Mr. M. SHAPAVALOV, who writes that "all were tested in inoculation experiments in 1912–1913, and proved to be pathogenic." Three of the other organisms were found to be identical morphologically with the one figured in plate VIII, while the fifth did not produce an aërial mycelium sufficiently profuse to permit of a satisfactory microscopic examination, although the general appearance of the culture indicated that it also is identical with *Actinomyces* XVI.

ACTINOMYCES XVIII

CULTURAL CHARACTERS.—On glucose agar, growth meager; nutritive mycelium colorless; aërial mycelium slow to develop, first white, later showing slight discoloration; diffusible pigment absent. On potato agar, development very rapid; nutritive mycelium dark; aërial mycelium profuse, felty, bluish gray; guttation moderate; tyrosinase reaction vigorous.

MORPHOLOGY.—This species is characterized by an unusual degree of variability in its fructifications. In figs. 102 and 107 is represented a relationship between axial filament and sporogenous branches common to many members of the genus. Fig. 108 shows a slight departure from this type in the thickening of the subterminal portion of the axial filament bearing the spiral branches. Further departures are expressed in the tufted grouping of the spiral hyphae in fig. 104, and in the distended and extremely vacuolated condition of the axial filament in fig. 106. A strikingly

aberrant type is seen in fig. 103, the fertile branches being short, inserted at close, irregular intervals, and showing no spiral tendency; while the axial filament is thick and abounding in spherical structures containing either deposits of metachromatic material or a partial equatorial septum.

In the dextrorse spiral hyphae of 1–8 open turns, 2.0–3.0 μ in diameter, the ellipsoidal spores, 0.8–0.9 × 1.0–1.6 μ, are produced by the insertion of conspicuous septa, sometimes in association with spherical structures. The presence of the latter (fig. 106), however, is not here indicated by local distensions. Subsequently the cross-walls undergo constriction and conversion to narrow connecting isthmuses. In the aberrant fertile hyphae (those without any spiral tendency), sporogenesis appears to take place in a more miscellaneous manner. Definite septa can rarely be distinguished, the spores seeming to result from protoplasmic contractions.

Isolated once from soil collected in Cambridge, Massachusetts.

Summary

1. The vegetative thallus of *Actinomyces* consists of a mycelium composed of profusely branching hyphae, the terminal growing portions of which are densely filled with protoplasm. Toward the center of the thallus the vacuoles increase in size and may be associated with the presence of metachromatic granules, the latter having nothing in common with bacterial endospores or "micrococci," for which they were mistaken by early observers.

2. The vegetative mycelium attains an extent incomparably greater than the branching figures recorded for bacteria of the acid-fast group, and the hyphae lack the uniformity in diameter generally characteristic of the Schizomycetes.

3. The aërial mycelium produced on suitable substrata by most species occurs usually in the form of a mat of discrete fructifications; but in other species these fructifications are frequently combined to form numerous and peculiar erect Isarioid sporodochia.

4. In any case each individual fructification represents a well characterized sporogenous apparatus, consisting of a sterile axial filament bearing branches in an open racemose or dense capitate arrangement. The primary branches may function directly as

sporogenous hyphae, or may proliferate branches of the second and of higher orders, sporogenesis in the latter case being confined to the terminal elements, the hyphal portions below the points of attachment of branches remaining sterile.

5. Two tendencies in the development of fructifications are recognizable: one leading to an erect drendroidal type, in which successively proliferated fertile elements undergo processes of sporogenesis in continuous sequence; and the other leading to a prostrate racemose type, in which sporogenesis is delayed in the older branches until the younger branches have also attained their final extension. The majority of species show these tendencies combined in different ways.

6. The sporogenous hyphae of most species are coiled in peculiar spirals, sometimes resembling the spores of the hyphomycetous genus *Helicoön*. These spirals exhibit pronounced specific characteristics in the number, diameter, and obliquity of their turns, and especially in the direction of rotation (whether dextrorse or sinistrorse).

7. Sporogenesis, where it can be followed, begins at the tips of the fertile branches and proceeds basipetally. In the larger number of species the process involves the insertion of septa which, in certain cases, are relatively very massive, and in others so thin as to be barely discernible. The disposition of these septa, while the delimited spores undergo maturation processes, varies with the species: (1) they may remain more or less unaltered; (2) they may suffer a median split, the two resulting halves being then separated as the result of the longitudinal contraction of the young spores, leaving alternate portions of hyphal walls completely evacuated; or (3) they may first become considerably constricted and subsequently converted into non-stainable isthmuses connecting the mature spores. The apparent absence of septa in the sporogenous hyphae of other forms is perhaps attributable to optical difficulties.

8. Granules are readily differentiated in the spores of many species which possess the staining properties and uniformity of size characteristic of nuclei; they generally occur singly, but in the larger spores of a few forms two are often found occupying diagonally opposite positions.

9. As in the vegetative thallus, metachromatic granules occur in the aërial mycelium, being very rarely found in the spores or sporogenous hyphae, but becoming very abundant in degenerate sterile hyphae.

10. The older axial filaments of some species show marked distensions which, in extreme cases, result in figures simulating *Leptomitus*. These arise as local distensions at the points of attachment of the more extensive lateral sporogenous processes. Cuneate modifications of the sterile axial filaments below the origins of branches also occur.

11. Curious spherical structures appear regularly in some forms, both in the sterile axial hyphae, where they may contain either a median septum or a number of peripheral metachromatic granules, and in the sporogenous hyphae, where they are associated with the regularly spaced septa.

12. The spores germinate readily in suitable solutions, producing 1–4 germ tubes, the approximate number being more or less characteristic of the species.

13. Owing to the absence of any well defined bacterial characteristics, the writer is of the opinion that the view that *Actinomyces* represents a transition between the Hyphomycetes and the Schizomycetes, as well as the phylogenetic corollary based upon it, may safely be abandoned. If mere size is to be regarded as important, it would appear to be equally profitable to look for bacterial affinities in some ascomycetous and sphaeropsideaceous forms, the hyphae of which are similarly very minute. It is doubtful whether far-reaching taxonomic generalizations can be based on the "acid-fast" staining reaction, especially as this reaction has not played a very important rôle in mycological research. There seems to be no adequate reason why the genus should not be classed in an unqualified manner with the Hyphomycetes, as a mucedineous group with tendencies toward an erect Isarioid habit.

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CRYPTOGAMIC LABORATORIES
HARVARD UNIVERSITY
CAMBRIDGE, MASS.

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EXPLANATION OF PLATES II–IX

All except figs. 7, 8, 70, and 101, which are semidiagrammatical representations with a magnification of approximately 8000, were drawn with the aid of a camera lucida with a magnification of 2750.

PLATE II

Actinomyces I

Fig. 1.—Moderately well developed fructification.

Fig. 2.—Somewhat smaller fructification showing successive order of development: *a*, chain of spores, largely disrupted, developed from termination of axial hypha; *b*, *c*, *d*, secondary branches that have given rise respectively to series of elements *b1–b4*, *c1–c5*, and *d1–d3*.

Fig. 3.—Spore germinating with 4 germ tubes.

Actinomyces II

Figs. 4–6.—Portions of aërial mycelium, showing conspicuous septa in fertile branches, and relation of latter to axial filaments: *a*, *b*, *c*, branches proliferated successively from same filament; *b1–b3*, *c1–c3*, elements proliferated successively from branches *b* and *c* respectively.

Fig. 7.—Portion of branch *c* (fig. 5) showing attachment of successively formed spiral elements.

Fig. 8*a–f*.—Successive stages in development of fertile hypha.

Actinomyces III

Figs. 9–14.—Portions of aërial mycelium.

Fig. 15.—Portion of degenerate hypha containing abundance of metachromatic material.

PLATE III

Actinomyces IV

Fig. 16.—Short chain of spores showing nuclei, and 2 deep staining remnants of constricted septa in hyaline isthmuses between spores.

Figs. 17–20.—Portions of aërial mycelium.

Fig. 28.—Spore germinating with 2 germ tubes.

Actinomyces V

FIG. 21.—Aërial hypha with spiral termination and 2 fertile branches, more mature elements showing failure of spore to develop in proximal portion.

FIG. 22.—Aërial hypha with 2 spiral elements.

FIG. 23.—Young spiral branch of 15 turns attached to axial hypha containing metachromatic granules.

FIGS. 24–26.—Spiral branches soon after insertion of septa, showing cross-walls absent from portion above basal septum.

FIG. 27.—Young spiral branch.

FIGS. 29, 30.—Spiral branches with spores mature, and non-septate portion completely evacuated.

FIGS. 31, 32.—Degenerate filaments containing much metachromatic material.

Actinomyces VI

FIG. 33.—Portion of aërial mycelium showing 2 spiral elements with nuclei in mature spores of one; septum in axial filament associated with basal septum in branch.

FIG. 34.—Similar to fig. 33, but without visible nuclei.

FIGS. 35–37.—Other portions of aërial mycelium.

Actinomyces VII

FIGS. 38–40.—Portions of aërial mycelium with sporogenous branches in various stages of development.

PLATE IV

Actinomyces VIII

FIG. 41.—Sporogenous apparatus with mature spores cohering in zoogloea-like masses.

FIG. 42.—Prostrate hypha containing numerous metachromatic granules and bearing a branch with many crowded spiral ramifications.

FIG. 43.—More open type of sporogenous apparatus with lateral elements attached to axial hypha at intervals.

FIG. 44.—Young sporogenous apparatus with spiral branches more or less crowded.

FIG. 45.—Somewhat older system of spiral hyphae, some of which have become converted into zoogloea-like masses of spores.

FIG. 46.—Lateral element bearing 8 spiral branches.

FIGS. 47, 48.—Portions of degenerate mycelium showing *Leptomitus*-like enlargements occupied by vacuoles, and metachromatic granules in constrictions.

FIGS. 49, 50.—Spores germinating with 1 germ tube.

Actinomyces IX

FIG. 51.—Portion of aërial mycelium showing spiral termination converted into chain of uninucleated spores, and presence of remnants of septa in hyaline isthmuses.

FIG. 52.—Similar to fig. 51, but without indications of septa between spores.

FIG. 53.—Portion of aërial mycelium showing septa in axial filament above insertions of some sporogenous branches.

FIG. 54.—Sporogenous branches with portion below second turn of spiral conspicuously thickened.

FIG. 55.—Sporogenous branch of 11 turns.

FIG. 56.—Spore germinating with 1 germ tube.

FIG. 57.—Sporogenous branch of 15 close spiral turns.

Actinomyces X

FIGS. 58–60.—Portions of aërial mycelium.

FIGS. 61, 62.—Spores germinating with 1 and 2 germ tubes respectively.

PLATE V

Actinomyces XI

FIGS. 63, 64.—More or less erect fructifications terminating long prostrate filaments, showing origin of groups of sporogenous branches from local hyphal distensions occupied by conspicuous vacuole.

FIGS. 65, 66.—Intermediate portions of aërial mycelium.

FIG. 113.—Spore germinating with 1 germ tube.

Actinomyces XII

FIGS. 67, 68.—Erect fructifications terminating long prostrate aërial filaments, exhibiting a pronounced tendency toward successive type of development, and showing cuneate hyphal enlargements below insertions of branches.

FIG. 69.—Intermediate portion of aërial mycelium.

FIG. 70*a–e*.—Progressive stages in development of sporogenous hypha, occurring in this and numerous other species.

FIG. 71.—Spore germinating with 3 germ tubes.

PLATE VI

Actinomyces XIII

FIG. 72.—Two portions, *a* terminal, *b* subterminal, of one long, unbranched, continuous sporogenous hypha showing very slight spiral tendency.

FIG. 73.—Chain of spores with deep staining polar granules.

FIGS. 74, 75.—Portions of aërial mycelium showing branching.

Actinomyces XIV

FIGS. 76–81.—Portions of aërial mycelium showing arrangement of sporogenous branches on hyphae, and method of sporulation.

PLATE VII

Actinomyces XV

FIG. 82.—Portion of aërial mycelium: elements *a1–a3* and *b1–b3* successively proliferated from branches *a* and *b* respectively.

FIG. 83.—Sporogenous branch *a* with 2 secondary branches; younger (*a3*) associated with a septum above insertion (successive type); older (*a2*) not set off by septum.

FIG. 84.—Axial filament with 3 branches, bearing successively poliferated elements *a1–a3*, as well as branch *ax*, latter not associated with septum in primary branch above point of attachment.

FIG. 85.—Fructification developed entirely in successive sequence, with 2 chains of uninucleated spores.

FIGS. 86–88.—Spores germinating with 2 germ tubes.

Actinomyces XVI

FIG. 89.—Large fructification consisting of axial filament *a–a1*, with whorl of 5 primary branches *a2–a6*, each bearing 1 or more secondary branches.

FIG. 90.—Spiral termination of sporogenous branch.

FIG. 91.—Old filament containing many metachromatic granules.

PLATE VIII

Actinomyces XVII

FIG. 92.—Portion of aërial mycelium showing spherical structures associated with septa in local distensions of sporogenous branch.

FIGS. 93, 94.—Portions of aërial mycelium, some lateral elements bearing secondary branches (indicated by numerals above 1) developed successively; 94*c*, unusually long fertile branch.

FIG. 95.—Portion of aërial mycelium similar to one shown in fig. 92.

FIGS. 96–100.—Spores germinating with 1 or 2 germ tubes.

FIG. 101*a–e*.—Successive stages in development of sporogenous branch, *cx* and *cy* representing either alternative or probably successive stages.

PLATE IX

Actinomyces XVIII

FIG. 102.—Sporogenous branch of usual type soon after appearance of septa.

FIG. 103.—Portion of fructification bearing aberrant fertile branches without spiral terminations.

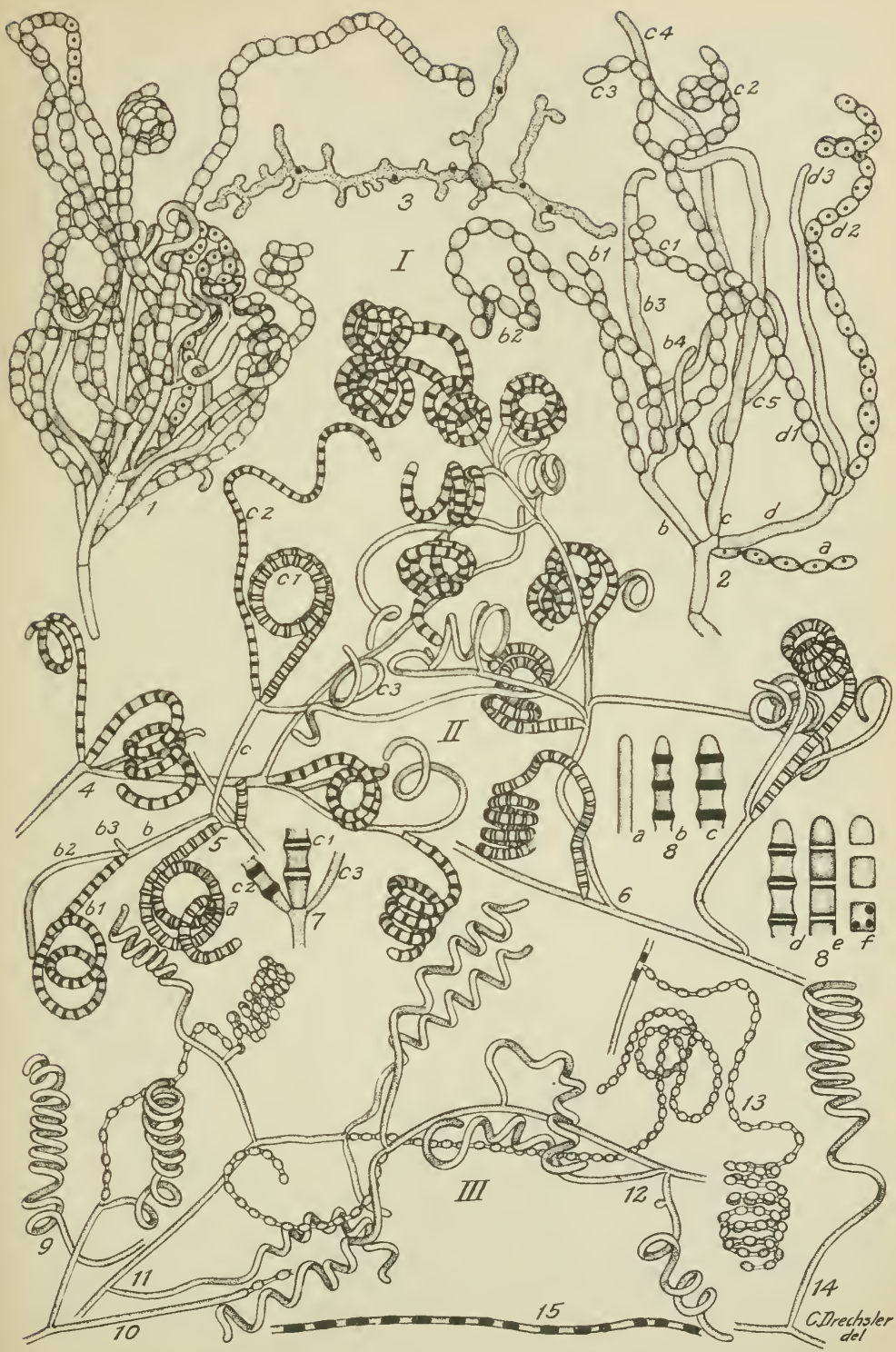
FIG. 104.—Aërial filament with several spiral branches borne terminally.

FIG. 105.—Chain of mature spores developed from branch of spiral type.

FIG. 106.—Degenerate axial filament containing large vacuoles and spherical structures and bearing a fertile spiral branch.

FIGS. 107, 108.—Portions of aërial mycelium showing fertile hyphae of spiral type.

FIGS. 109–112.—Spores germinating with 1 or 2 germ tubes.



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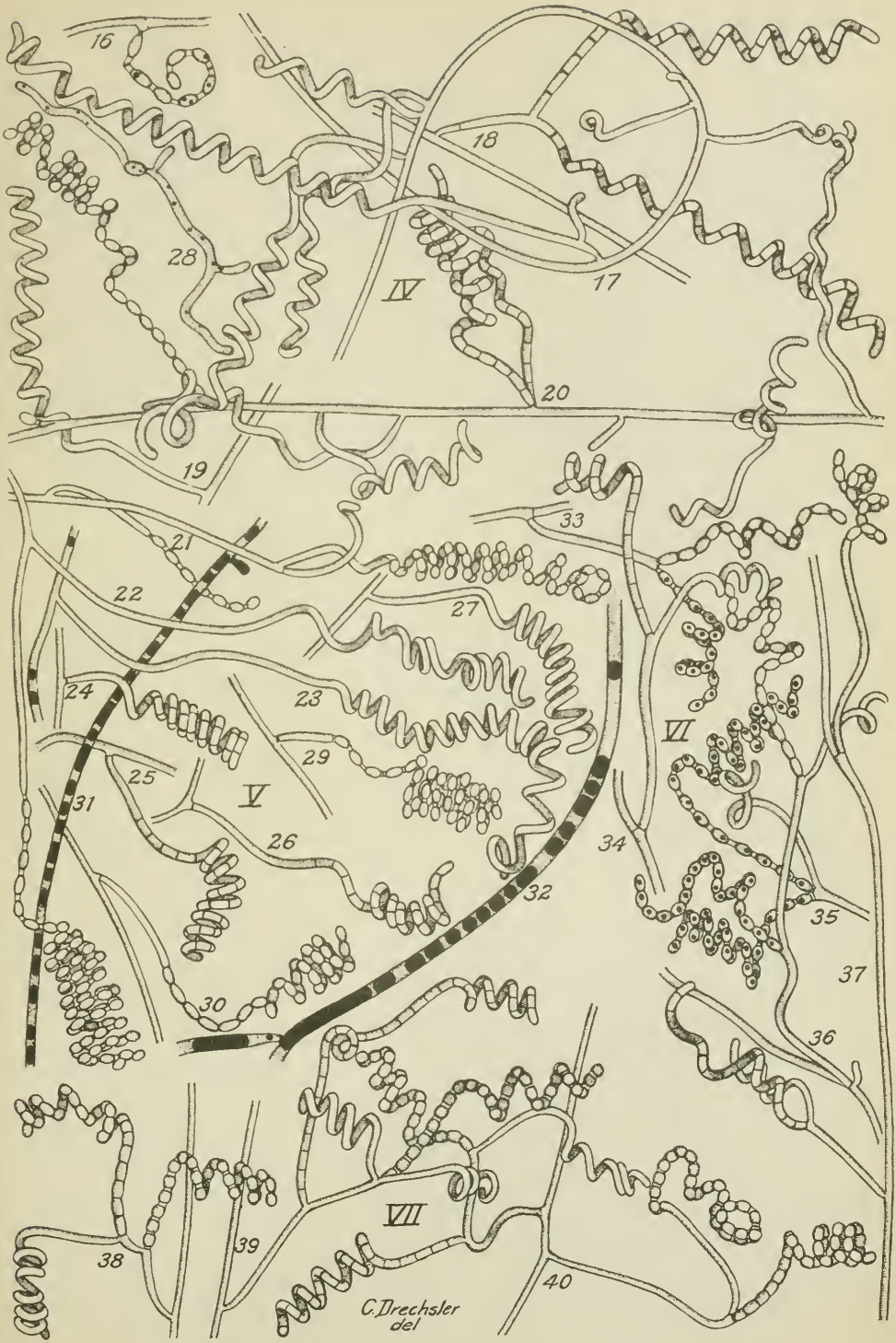

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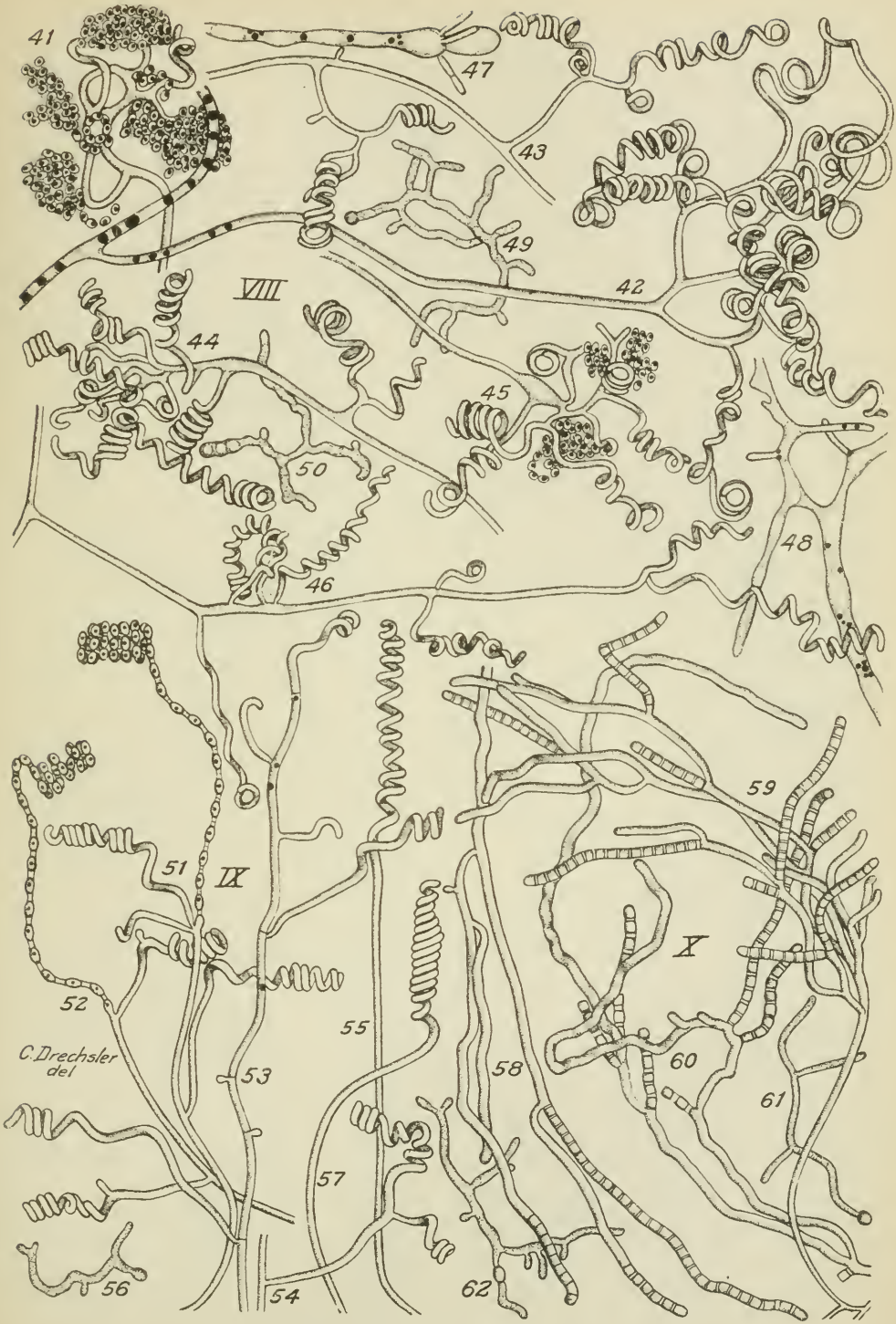

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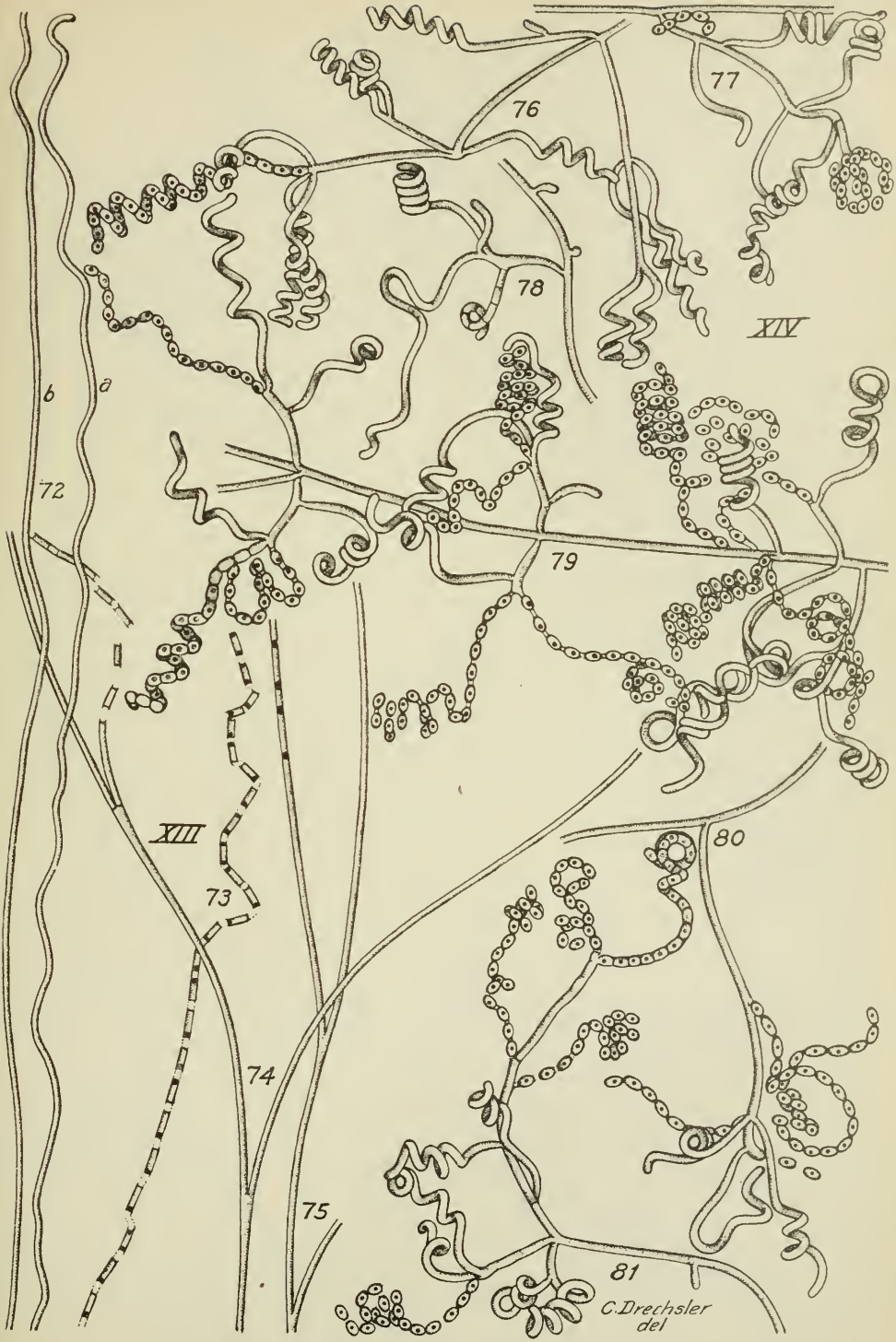

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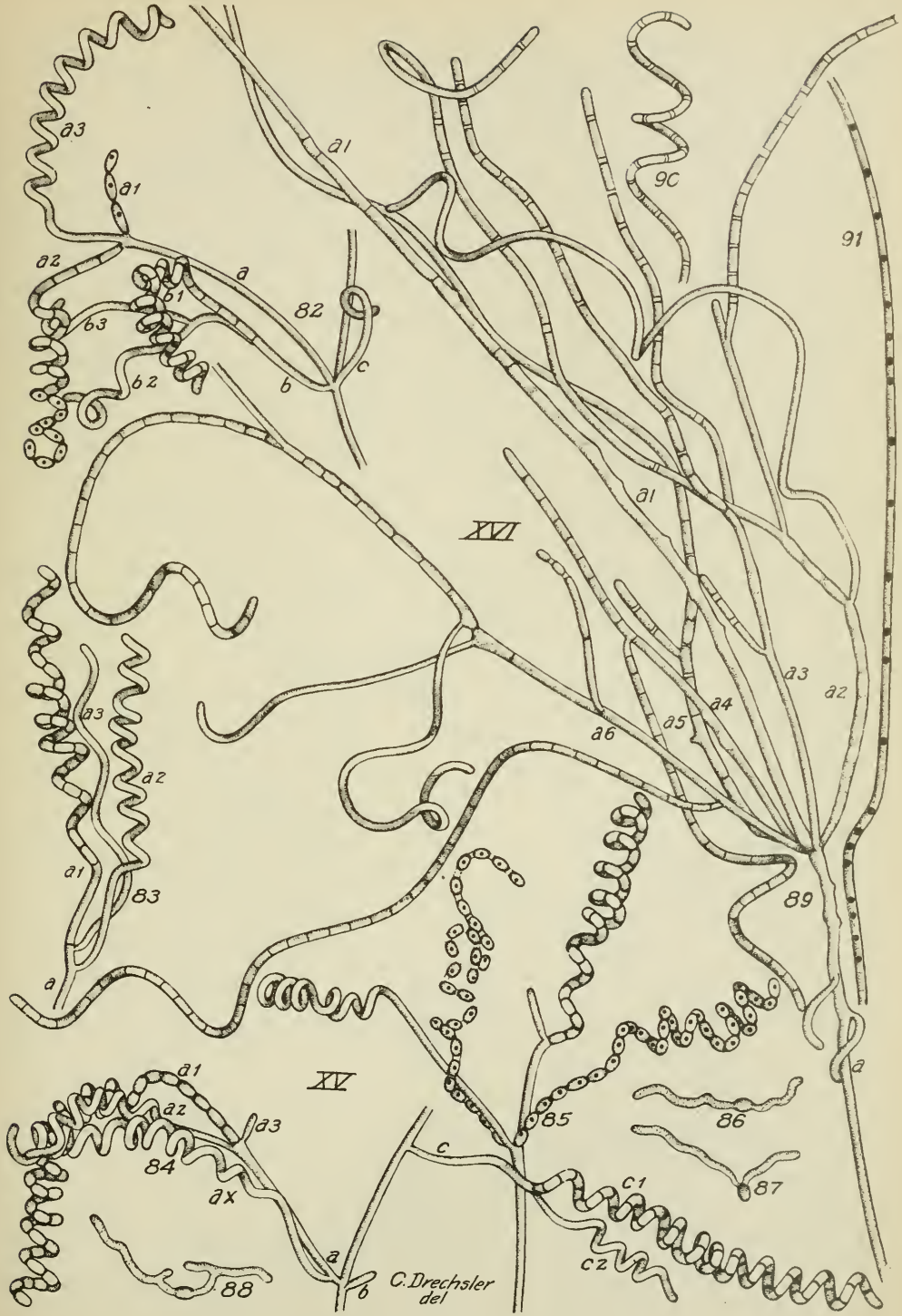

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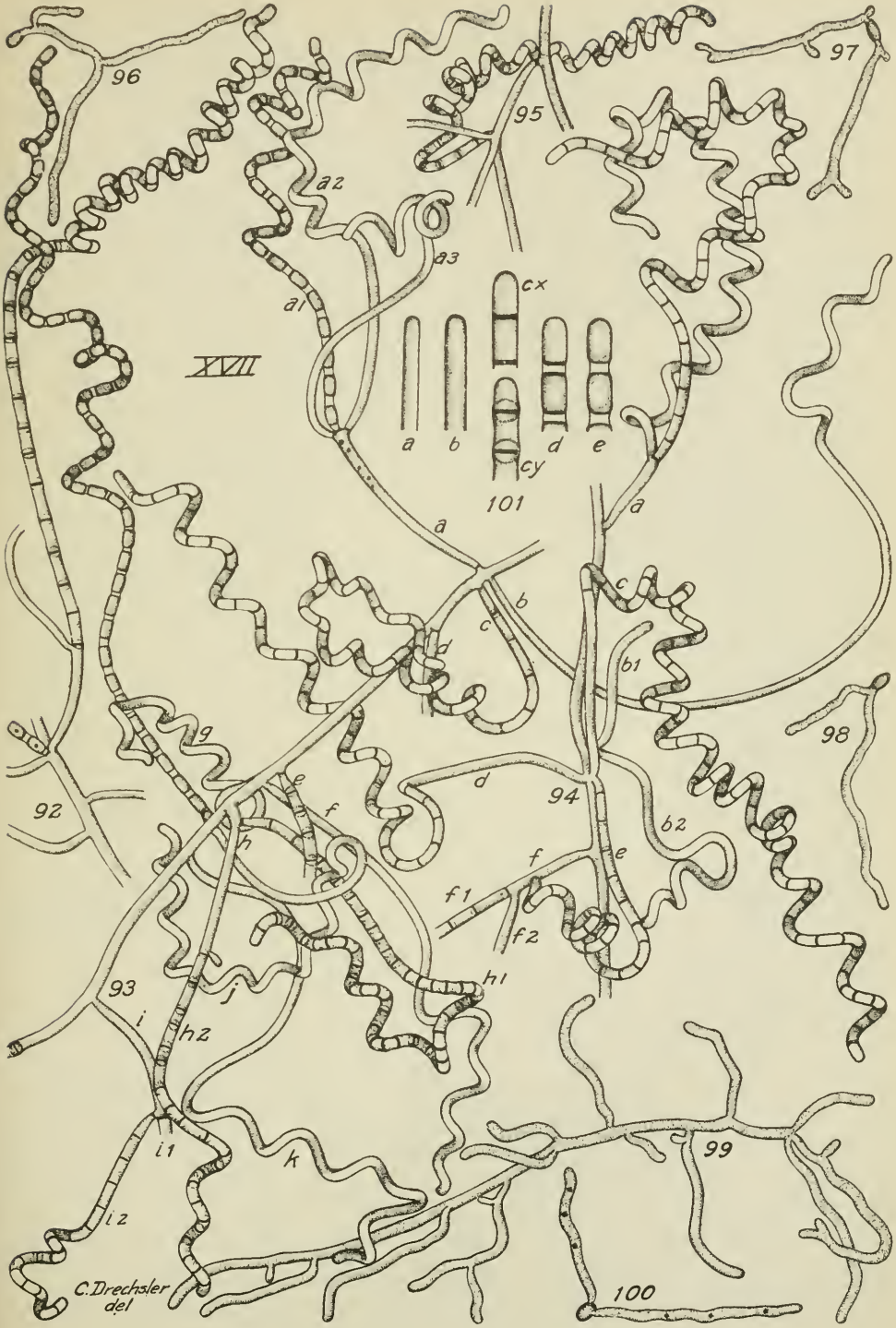

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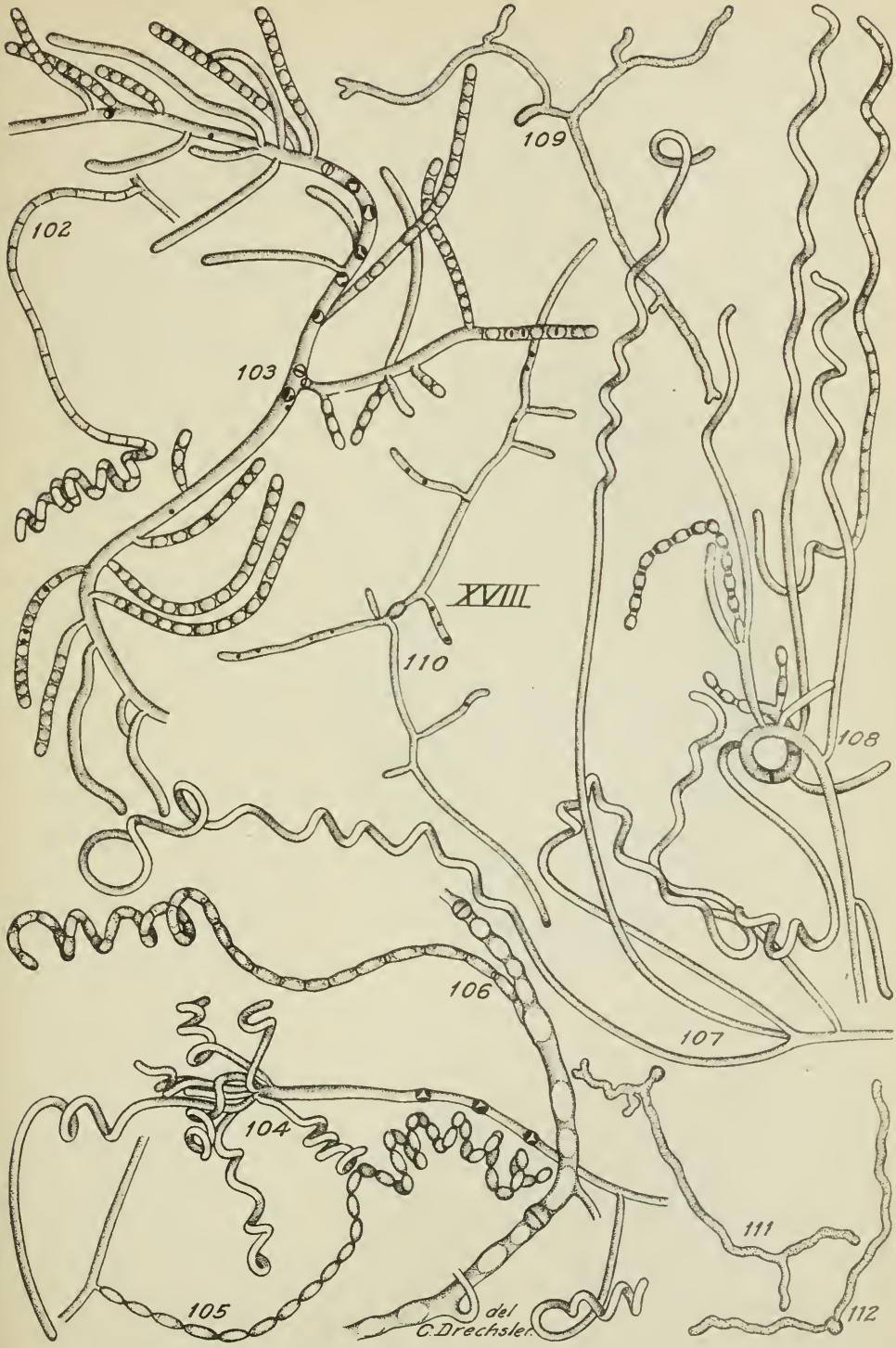

DRECHSLER on ACTINOMYCES

BRIEFER ARTICLES

BYRON DAVID HALSTED

(WITH PORTRAIT)

With the passing of BYRON DAVID HALSTED we have lost another of our pioneer botanists. Although not one of the earliest pioneers, he was a pioneer in many respects. He was one of that group of botanists who laid the foundation of the science in America at a time when the subject was recognized by very few American colleges and universities. He was one of that still smaller group who took up the study of applied botany and worked faithfully for its advancement.

Our younger plant pathologists know how difficult it is to find a disease of an economic crop that is not at least mentioned in his reports. He was among the first to report the introduction of several foreign pathogenic organisms.

Born at Venice, Cayuga County, New York, June 7, 1852, he was left an orphan at an early age and was cared for by relatives. He graduated from the Michigan Agricultural College with the degree of B.S. in 1871, and received the M.S. degree from the same college in 1874. In 1878 he received the Sc.D. degree from Harvard, being the first man to take the doctorate in cryptogamic botany from that university. He was managing editor of the *American Agriculturist* from 1880 to 1885; Professor of Botany in the Iowa Agricultural College 1885–1889; and Professor of Botany in Rutgers College

and Botanist of the New Jersey Agricultural Experiment Station from 1889 until his death, August 26, 1918. Had he lived until February, 1919, he would have rounded out a full 30 years in the service of the state of New Jersey. During the greater part of these 30 years he was active in both College and Station, but in the latter part of his career poor health necessitated his retirement from the classroom.

Although a very busy man, he found time to serve his science by acting as Associate Editor of the *Bulletin of the Torrey Botanical Club* from 1890 to 1893 and as a contributor to the *Systematic Flora of North America*. In 1877 the Massachusetts Horticultural Society honored him with its silver medal. He was a member of a number of scientific societies, serving as president of the Society for the Promotion of Agriculture from 1877 to 1879 and of the Botanical Society of America in 1900–1901.

Dr. HALSTED was a true lover of nature, and nature made him a most warm hearted and lovable man. He loved to commune with nature and was a most enthusiastic collector. In addition to his own studies, he furnished a great quantity of material for study by other mycologists and from which many new species were described; in fact, the mycological collections not only of America but of the entire world contain material collected by him.

The writer looked upon him as a botanist of the old school, and yet he was an up-to-date botanist in every way. After devoting the greater part of his career to mycology, poor health and a failing eyesight forced him to abandon his favorite line of work. He could not leave the field of botany, however, but merely transferred his efforts to a line of plant breeding which did not require the use of the microscope, and worked with the renewed energy and the enthusiasm of a boy.

Dr. HALSTED was more than a botanist; he was a broad, scholarly man and a public spirited citizen. He was always interested in athletics and in his youth was an amateur baseball pitcher. He never lost his interest in the sport, but was a regular attendant at intercollegiate games, always placing himself so that he could observe the work of the pitcher. His love for literature and his keen interest in the state and community were made manifest by a poem which he wrote on the occasion of a civic parade when the nation was called to arms in 1917.

He was the author of many papers, and while most of us will think of him as a scientist, it should be remembered that many of his papers had to do with other subjects.—MEL. T. COOK, *New Jersey Agricultural Experiment Station, New Brunswick, N.J.*

CURRENT LITERATURE

BOOK REVIEWS

Soil conditions and plant growth

The rapid progress that is being made in the scientific field encompassed by plant physiology and soil science is evidenced in the appearance of a third and enlarged edition of RUSSELL'S[1] *Soil conditions and plant growth* in the series of Monographs on Biochemistry edited by PLIMMER and HOPKINS. The first edition (1912) contained 166 pages, including 13 pages of citations; the second edition (1915) contained 188 pages, including 14 pages of citations; and the third edition (1917) contains 240 pages, including 18 pages of citations. The growth of the subject is marked by the addition of a chapter on "The relationship between the micro-organic population of the soil and the growth of plants" to the second edition, and a chapter on "The colloidal properties of soil" to the third edition. In his preface to this new edition the author states that he has not attempted to refer to every paper published on the subject since the first edition, but that his "guiding principle has been to include only those that brought in some new idea or profoundly modified an old one." The choice of papers is naturally a question of judgment, yet the reviewer feels that certain important omissions have been made, and he will therefore direct the reader's attention to these in the hope that they may serve to supplement this admirable treatment of practical plant physiology. Certain papers published since 1917, and therefore not referable to as "omissions," will be included in order to bring the subject up to date.

Chapter II on "The requirements of plants" presents a modern physiological basis for the rest of the book. This chapter has been enlarged to the extent of 5 pages over the first edition. In the discussion of limiting factors the treatment of oxygen (only 9 lines) and temperature seems quite inadequate. Attention is therefore directed to the following papers:

On temperature relations of plants.—LEITCH, I., Some experiments on the influence of temperature on the rate of growth of *Pisum sativum*. Ann. Botany 30:25–46. 1916; LEHENBAUER, P. A., Growth of maize seedlings in relation to temperature. Physiol. Researches 1:247–288. 1914; LEPESCHKIN, W. W., Zur Erkenntnis der Einwirkung supramaximaler Temperaturen auf die Pflanze. Ber. Deutsch. Bot. Gesell. 30:703–714. 1912; MAXIMOW (MAKSIMOV), N. A., Chemische Schutzmittel der Pflanzen gegen Erfrieren. Ber. Deutsch. Bot.

[1] RUSSELL, E. J., Soil conditions and plant growth. 3d ed. pp. 243. *figs. 14.* New York: Longmans, Green and Co. 1917.

Gesell. 30:52–65, 293–305, 504–816. 1912; GROVES, J. F., Temperature and life duration of seeds. BOT. GAZ. 63:169–189. 1917.

On oxygen relations of plants.—SHULL, CHARLES A., The oxygen minimum and the germination of *Xanthium* seeds. BOT. GAZ. 52:453–477. 1911; CANNON, W. A., and FREE E. E., The ecological significance of soil aëration. Science N.S. 45:178–180. 1917; LIVINGSTON, B. E., and FREE, E. E., The effects of deficient soil oxygen on the roots of higher plants. Article in "Contributions to Plant Physiology." Johns Hopkins University. Reprint from Johns Hopkins University Circular. March 1917.

The new edition deserves high praise for its comprehensive treatment and impartial judgment on the modern developments in soil chemistry. The discussion on the use of dilute acids in soil analysis, based on the author's own work in the Rothamsted laboratories, is the first contribution on this subject that has forsaken empirical experimental methods and adopted a modern physico-chemical procedure. The review of the highly controversial literature on soil acidity is eminently fair. The attention of the reader is called to the following recent papers, each of which contains an extensive bibliography: CHRISTENSEN, H. R., Experiments in methods for determining the reaction of soils. Soil Science 4:115–178. 1917; TRUOG, E., Soil acidity. I. Its relation to the growth of plants. Soil Science 5:169–195. 1918; RICE, F. E., and OSUGI, S., The inversion of cane sugar by soils and allied substances and the nature of soil acidity. Soil Science 5:333–358. 1918.

The biological aspects of soil science are fully treated, including the author's own interesting ideas on soil protozoa and partial sterilization. The reviewer feels that RUSSELL has been somewhat partial to his own views in not referring to SHERMAN'S studies. SHERMAN concludes, on the basis of his experiments, that "no evidence has been obtained which indicates that the beneficial effect of partial sterilization is due to the elimination of a biological factor which is harmful to the bacteria." BOLLEY'S interesting views on "Soil sanitation" deserve mention in a chapter that bears such an all-inclusive title as "The relationship between the micro-organic population of the soil and the growth of plants." The following papers should be read in connection with the chapter: SHERMAN, J. M., Studies on soil protozoa and their relation to the bacterial flora. Jour. Bacteriology 1:35–66, 165–185. 1916; KOPELOFF, N., and COLEMAN, D. A., A review of investigations in soil protozoa and soil sterilization. Soil Science 3:197–269. 1917; BOLLEY, H. L., Wheat-soil troubles, causes of soil sickness, etc. Bull. 107. N.D. Agric. Exper. Sta. 1913; BOLLEY, H. L., Conservation of the purity of soils in cereal cropping. Science N.S. 32:529–541. 1910; BOLLEY, H. L., Cereal cropping: sanitation, a new basis for crop rotation, manuring, tillage, and seed selection. Science N.S. 38:249–259. 1913; HOPKINS, C. G., The bread supply. Science N.S. 38:479–481. 1913.

KEEN'S mathematical studies on the retention of water by soil are amply discussed, but no mention is made of SHULL'S even more important contribution to the problem of the wilting coefficient. The reader is therefore referred to the

following: SHULL, C. A., Measurement of the surface forces in soils. BOT. GAZ. 62:1–31. 1916.

The final chapter is devoted to a theoretical discussion of soil analysis. An appendix describes analytical methods used in England. The reviewer believes that the reader should be cognizant of the following discussions of American methods: BEAR, F. E., and SALTER, R. M., Methods in soil analysis (Technical Bulletin). Bull. 159. West Virginia Agric. Exper. Sta. Morgantown. 1916; AMES, J. W., and SCHOLLENBERGER, C. J., Liming and lime requirement of soil. Bull. 306. Ohio Agric. Exper. Sta. Wooster. 1916; TRUOG, E., A new test for soil acidity. Bull. 249. Wisconsin Agric. Exper. Sta. Madison. 1915; BOUYOUCOS, GEO. J., and MCCOOL, M. M., The freezing point method as a new means of measuring the concentration of the soil solution directly in the soil. Tech. Bull. 24. Michigan Agric. Exper. Sta. East Lansing. 1915.—H. L. WALSTER.

MINOR NOTICES

History of phytopathology.—WHETZEL,[2] in his *History of phytopathology*, aims "only to set forth in outline what appear to be the most outstanding features in the evolution of the science, and to indicate the proper relation thereto of the men who have chiefly shaped its progress." The chief captions are: (1) The Ancient Era, to the end of the 5th century (5 pp.); (2) The Dark Era, 6th to 16th centuries (1 p.); (3) The Premodern Era, 1600 to about 1850 (19 pp.); (4) The Modern Era, 1853 to about 1906 (65 pp.); (5) The Present Era, 1906 (8 pp.). As is indicated by the page allotment, the first and second topics are treated very briefly, being barely sketched. The third and fifth topics are treated somewhat more fully, while the most page space is given to "The Modern Era." The book is in the main a series of brief biographical sketches, often with portraits, arranged chronologically under the captions indicated. It will be a convenient reference book for those who may need ready access to such biographies.—F. L. STEVENS.

Winter botany.—To supplement his pocket manual of woody plants, already noted in this journal,[3] TRELEASE[4] has compiled and published a companion volume for use in naming our common trees and shrubs when without foliage. The range, extending to 326 genera and over 1000 species, includes most introduced as well as native woody plants. The notable features of the volume, aside from its convenient pocket size and abundant illustrations from most accurate drawings, are the numerous keys and the many citations of literature dealing with winter characters of the various genera and species. The

[2] WHETZEL, H. H., An outline of the history of phytopathology. pp. 130. Saunders Co. 1918.

[3] BOT. GAZ. 65:194. 1918.

[4] TRELEASE, WILLIAM, Winter botany. 16mo. pp. 394. *figs. 327*. Urbana, Ill. Published by the author. 1918. $2.50.

drawings are of bud, leaf-scar, pith, and other twig characters upon which the keys are based, so that with the use of a hand lens it should be possible to determine readily the genera, and for the most part the species, for native and introduced trees and shrubs. The author is to be congratulated in making such a fund of unusual information available in such a compact and readily available form.—GEO. D. FULLER.

American trees.—Another book on trees, by EMERSON and WEED,[5] has been added to the already large number upon the same subject. It is essentially a book for the amateur, since its chief virtue lies in the excellent photographs by EMERSON, an entire page being devoted to each species. The absence of keys of any sort renders the book comparatively useless for the identification of an unknown species, but the quality and abundance of the illustrations will make it one the tree lover will wish to have upon his table.—GEO. D. FULLER.

NOTES FOR STUDENTS

Physiological balance in soil and other nutrient solutions.—HIBBARD[6] has just published a piece of work on physiological balance in soil solution which is to mark a decided advance (both theoretically and practically), if the future development of the work approximates its present promise. He extracted the soil solution from an infertile very sandy soil and from a fertile sandy loam by the Van Suchtelen oil pressure method as improved and extended in usefulness by MORGAN.[7] HIBBARD speaks of this as giving a more concentrated solution than any other extraction method. The solution thus extracted from the poor sandy soil had an osmotic pressure of 0.193 atmospheres, and that from the good soil 1.81 atmospheres. The soil extracts showed an order of production similar to the soils from which they came.

The soil extracts were used instead of distilled water to prepare the Shive 3-salt (KH_2PO_4, $Ca(NO_3)_2$, $MgSO_4$) nutrient solution. The total concentration of nutrient salts added gave an osmotic pressure of 1.75 atmospheres, and in the 36 different solutions made up from each soil extract and from distilled water the proportions of each salt varied from 10 to 80 per cent of the total nutrient salt osmotic concentration.

In the nutrient solution made from the extract of the poor soil the optimum osmotic proportions of the KH_2PO_4, $Ca(NO_3)_2$, and $MgSO_4$ for the growth of Fultz wheat were 7:1:2 respectively, with a total osmotic pressure of (1.75+0.193) 1.94 atmospheres; in that made from the extract of the good soil 2:7:1 respectively, with a total osmotic pressure of (1.75+1.81) 3.56 atmospheres; and in that made with distilled water 5:2:3, with a total osmotic pressure of

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[5] EMERSON, ARTHUR I., and WEED, C. M., Our trees, how to know them. New ed. pp. xxi+295. *pls. 149*. Philadelphia: Lippincott Co. $3.50.

[6] HIBBARD, R. P., Physiological balance in the soil solution. Tech. Bull. Mich. Agric. Exper. Sta. no. 40. pp. 44. 1917.

[7] Tech. Bull. Mich. Agric. Coll. Exp. Sta. no. 28.

1.75 atmospheres. Analyses of the soil extract of the poor soil showed it to be proportionally low in K_2O and P_2O_5. Also addition of KH_2PO_4 to the poor soil at the rate of 150–300 pounds to the acre greatly increases the growth of wheat. A similar analysis of the soil extract of the good soil showed it to be as poor in nitrates, nitrites, and ammonium salts as the extract from the poor soil, but with a relatively high calcium magnesium ratio. Addition of $Ca(NO_3)_2$ to the good soil at the rate of 200–400 pounds to the acre greatly improved the growth of the plants. HIBBARD concludes that the soil is deficient in calcium and perhaps nitrates. If, as HIBBARD'S results seem to indicate, one can tell the fertilizer needs of a soil by such a study of the soil extracts, he has given us a new practical method of great significance. He has also hinged the fertilizer question largely on the balance between nutrient ions.

HIBBARD worked only on the early stage of the growth of wheat. It would be of interest to know whether the same ratios apply to the middle and late stages. It will also be interesting to see the nature of these ratios when plants with high sulphur demands (alfalfa, cabbage, etc.), those with high potash demands (potato, tobacco, etc.), or those with high general nutrient demands (squash, cucumber, etc.) are used. These stand in contrast with the wheat with its moderate demands. The method also needs the test of a great range of soil types of various degrees of fertility. Considering all of these variables, the application of the method may become rather complex. The work is sure to stimulate a great amount of investigation.

The use of a 3-salt nutrient solution requires that the variation in concentration of the several nutrients shall always be in pairs. In Shive's solution a change in concentration of sulphur is always accompanied by a corresponding change in the concentration of magnesium, potassium by phosphorus, and calcium by nitrogen. There is no reason for thinking that the magnesium demands of a plant are thus tied up with the sulphur demands; in fact, in soils we think of the sulphur as existing mainly as calcium sulphate, and in fertilizer practice it is more generally added in this form. This is not an adverse criticism of the use of 3-salt nutrient solutions; it is rather an acknowledgment of the complexity and innate difficulties of the problem. Since so many variations in proportions of nutrients are possible, the investigator must study only a portion of these, if he completes his investigations within a reasonable time.

LIVINGSTON and TOTTINGHAM[8] recognize this shortcoming of the 3-salt nutrient solution when they mention the 6 possible combinations of salts and proceed to investigate the best proportions in the second combination. The possible combinations are:

I	II	III	IV	V	VI
$Ca(NO_3)_2$	$Ca(NO_3)_2$	$Ca(H_2PO_4)_2$	$Ca(H_2PO_4)_2$	$CaSO_4$	$CaSO_4$
KH_2PO_4	K_2SO_4	KNO_3	K_2SO_4	KNO_3	KH_2PO_4
$MgSO_4$	$Mg(H_2PO_4)_2$	$MgSO_4$	$Mg(NO_3)_2$	$Mg(H_2PO_4)_2$	$Mg(NO_3)_2$

[8] LIVINGSTON, B. E., and TOTTINGHAM, W. R., A new 3-salt nutrient solution for plant cultures. Amer. Jour. Bot. 5:337–346. 1918.

They grew Fulcaster wheat for 18 days in 12 different molecular proportions of the salt combination II. The nutrient solutions had a total osmotic pressure of 1.75 atmospheres. Of the 12 volume molecular proportions tried the following proved best: KNO_3 0.0216, $CaH_2(PO_4)_2$ 0.0026, $MgSO_4$ 0.0150. This finding must not be taken too seriously, however, for it is based on the extremely slim experimental evidence of a single set of cultures with 6 seedlings in each concentration; also very different molecular proportions (KNO_3 0.0036, $CaH_2(PO_4)_2$ 0.0078, $MgSO_4$ 0.0300) gave almost as good results as this optimum and very much better results than the intermediate combinations.

Using combination I (Shive's solution) with a total osmotic pressure of 1.75 atmospheres, SHIVE and MARTIN[9] have determined the optimum volume molecular proportions for the development of buckwheat during its early period of growth (24 days) and during its period of maturity. The following table gives the results based on maximum production of dry weight:

			Volume-molecular partial concentration			Yield of tops, roots, seeds relative to those from	
			KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$	Knop's solution taken as 1.00	Tottingham's solution taken as 1.00
Buckwheat grown to flowering (early growth period)	Water cultures	Tops	0.0144	0.0052	0.0200	1.61	1.33
		Roots	0.0144	0.0052	0.0200	1.58	1.27
	Sand cultures	Tops	0.0144	0.0052	0.0200	1.38	1.25
		Roots	0.0180	0.0104	0.0050	1.47	1.16
Buckwheat grown to maturity (late growth period)	Water cultures	Tops	0.0108	0.0130	0.0100	1.40	1.35
		Roots	0.0108	0.0130	0.0100	1.50	1.39
		Seeds	0.0108	0.0078	0.0200	1.28	1.74
	Sand cultures	Tops	0.0108	0.0130	0.0100	1.40	1.26
		Roots	0.0108	0.0130	0.0100	1.27	1.26
		Seeds	0.0108	0.0130	0.0100	1.24	1.17

It is interesting to see SHIVE's solution far superior to KNOP's and to TOTTINGHAM's for growth of buckwheat. In former work by SHIVE the maximum growth of wheat tops appeared in the following molecular proportions: KH_2PO_4, 0.0180; $Ca(NO_3)_2$, 0.0052; $MgSO_4$, 0.0150. The $Ca(NO_3)_2$ needs for buckwheat rises greatly in the late growth period, while the KH_2PO_4 and $MgSO_4$ requirements both fall.

Many of the excellent methods used in these 3-salt nutrient solution studies were worked up by MCCALL. MCCALL and RICHARDS[10] summarize their

[9] SHIVE, J. W., and MARTIN, W. H., A comparison of the salt requirements for young and for mature buckwheat plants in water cultures and sand cultures. Amer. Jour. Bot. 5:186–191. 1918.

[10] MCCALL, A. G., and RICHARDS, P. E., Mineral food requirements of the wheat plant at different stages of its development. Jour. Amer. Soc Agron. 10:127–134. 1917.

results on wheat in the following table, showing the mean molecular proportions of the 3 component salts and the ionic ratios for the culture solutions giving the best and the poorest growth of wheat during the different periods of development. They promise a full discussion of data with the publication of

Series and growth rank		Mean molecular proportions in tenths of total concentration		
		KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$
Series 1 first period	Best 9	3.4	4.7	1.9
	Poorest 9	3.8	2.0	4.2
Series 2 second period	Best 9	3.5	4.6	1.9
	Poorest 9	2.7	1.5	5.8
Series 3 third period	Best 9	2.0	5.5	2.5
	Poorest 9	6.1	1.8	2.1

results of experiments, now being carried on at the Maryland Experiment Station on the nutrient requirements of soy beans and buckwheat.

All this work indicates that Sachs, Knop, and others had not nearly exhausted the subject of desirable nutrient solutions for water and sand cultures, but that we still have much to learn. The concentration of the nutrient salts used in these solutions are far above those existing in the general soil solution, and upon the whole the immediate significance of this work in problems of soil fertility is not evident. In the soil the dissolving power of well developed root hairs in contact with nutrients of low solubility introduces a new and important feature. It must be stated, however, that Hibbard's work offers indications of an important bearing of this method on questions of soil fertility.—Wm. Crocker.

Respiration and age of plant organs.—Nicolas[11] has studied the respiration of very young leaves and leaflike structures in comparison with that of the corresponding fully developed organs taken from older parts of the same plants. In a few cases he included also a comparative study of the respiration of sections of the stem or branch bearing the young and old leaves. His material was selected from 15 different species, including annuals, biennials, and perennials. The studies were conducted by the method of confined atmospheres in absence of light at temperatures from 15 to 24° C., the gas analysis being made with the Bonnier-Mangin apparatus. Respiratory intensities were calculated on the basis of the fresh weight of the materials used. The "internal respiration" also was determined in a few cases, using hydrogen atmospheres. Respiratory quotients CO_2/O_2 and the ratio I/N between "internal respiration" and normal

[11] Nicolas, G., Contribution a l'étude des variations de la respiration des végétaux avec l'age. Rev. Gen. Botanique **30**: 214–225. 1918.

respiration are given, the former varying from 0.58 to 1.06 and the latter from 0.13 to 1.38.

In every case the young organ, whether leaf, cladode, or branch, had a greater respiratory intensity, a larger respiratory coefficient, and a lower intramolecular respiration than the corresponding older organ. This is true whether very young organs or organs in a somewhat later stage of development are compared with fully developed organs of the same year's growth or fully developed organs of the current year's growth are compared with organs of the previous year's growth (leaves of *Olea europea* L.).

The magnitude of the differences in respiratory intensity between young and old organs varied rather widely in the different species studied, and was evidently related to the relative differences in age. In general the respiratory intensity of the young organs was from 3 to 7 times that of the older organs.

The author reviews the work of previous investigators, all of whom agree that the respiratory intensity of young organs is greater than that of the corresponding older organs. Especially interesting in this connection are the studies of BONNIER and MANGIN,[12] of MAIGE,[13] and of Mme MAIGE,[14] as cited by NICOLAS in his article. BONNIER and MANGIN found two maxima for respiratory intensity in the seasonal development of a plant, one at the opening of the leaf buds or at the germinative period, the other at the time of flowering. MAIGE found that, while the respiratory intensity of flowers decreases with age when calculated on either wet or dry weight, it increases when stated in terms of amount of gaseous exchange per individual flower, and Mme MAIGE pointed out a decrease in respiratory intensity in each organ of the flower except the gynecium, where it sometimes increases with age.

The author gives reasons why it is thought that the respiratory differences observed between young and old organs cannot be explained by the absence of well developed cuticle in very young organs, the relative amounts of chlorophyll in the tissues, or the greater acidity of young organs, and raises the question whether they may not be referred to the activities of diastase and oxidase. Finally, the author refers to a previous paper with Mme MAIGE,[15] in which it was shown that increase in turgescence increases both the respiratory quotient and the respiratory intensity, and concludes that the turgescence of young

[12] BONNIER, GASTON, and MANGIN, LOUIS, Recherches sur les variations de la respiration avec le développement des plantes. Ann. Sci. Nat. Bot. VII. 2:315–364. 1885.

[13] MAIGE, M. A., Recherches sur la respiration de la fleur. Rev. Gen. Botanique 19:1–28. 1907.

[14] MAIGE, Mme G., Recherches sur la respiration des différéntes piéces florales. Ann. Sci. Nat. Bot. IX. 14:1–62. 1911.

[15] MAIGE, Mme A., and NICOLAS, G., Recherches sur l'influence des variations de la turgescence sur la respiration de la cellule. Rev. Gen. Botanique 22:409–422. 1910.

growing organs is of importance in determining the character and amount of their respiration.

The author's conclusions are as follows: "In young organs, principally leaves, intramolecular combustions are more complete than in older organs; young tissues consume much more oxygen than those completely developed, fix relatively less, and thus set at liberty greater quantities of energy, which they use in growth."—G. T. HARRINGTON.

Catalase, respiration, and vitamines.—DUTCHER[16] finds that the catalase activity of polyneuritic pigeons is very low, and that it rises to normal when the fowl is fed water soluble vitamine. His results are given in the following table:

CATALASE ACTIVITY OF TISSUES

Tissue	Polyneuritic pigeons, percentage of normal	Polyneuritic pigeons receiving water soluble vitamine, percentage of normal
Liver	44	110
Kidney	53	102
Pancreas	33	115
Heart	34	86
Breast	13	152
Lung	57	84
Blood	75	56
Average	44	101

The author says: "It is probable that polyneuritis is accompanied by incomplete or partial oxidation, with accumulation in the tissues of products of incomplete oxidation. It is also probable that water soluble vitamines function, directly or indirectly, in stimulation of oxidation processes, thereby clearing the tissues of toxic materials. When pigeon tissues are arranged in the order of their catalase content (as measured by the oxygen liberated from hydrogen peroxide), tissues group themselves in the order of their metabolic activity and also in the order of their content of water soluble vitamine."

APPLEMAN[17], in a recently published article, says: "Respiration in sweet corn in the milk stage is very high when the corn is first pulled. This high rate of respiratory activity falls off rapidly with storage. Catalase activity in a collateral set of ears showed a decline with storage, which is almost directly proportional to the decline in respiratory intensity after a like period of storage. The catalase activity of the expressed juice from both sweet corn and potato

[16] DUTCHER, R. ADAMS, Vitamine studies. I. Observations on the catalase activity of tissues in avian polyneuritis. Jour. Biol. Chem. **36**:63–72. 1918.

[17] APPLEMAN, C. O., Respiration and catalase activity in sweet corn. Amer. Jour. Botany **5**:207–209. 1918.

tubers is a fair index of the comparative intensity of respiration in the tissues. The data from both plant and animal tissues available at the present seem to justify the general indication that catalase action is invariably correlated with the oxidative processes involved in respiration."—Wm. Crocker.

Respiration of stored wheat.—Bailey and Gurjar[18] have done an excellent piece of work on the respiration of stored wheat. Significant literature is well presented and related to the work in hand, and the methods used in the work are clean cut and exact. The contribution has a very important application in the shipping and storage of grains. They worked with moisture contents ranging from 12 to 18 per cent, such as appear in the practical handling of grains. The following are the more important results.

Respiration gradually and fairly uniformly rises with moisture content up to 14.5 per cent in case of plump spring wheat. With the rise of moisture above this percentage the respiration is markedly accelerated. The soft starchy wheats respire more rapidly than the hard vitreous wheats containing the same percentage of moisture. With more than 14 per cent moisture shriveled wheat respires 2 to 3 times as fast as plump wheat of the same water content, due to a larger percentage of embryo in the shriveled grains; with less than 14 per cent moisture there is little difference.

Freshly dampened wheat respires more slowly than wheat of the same water content that has been dampened for a long time or that has been naturally dampened. The difference is noticeable at 13 per cent moisture, and rises as the moisture rises. Wheat stored at room temperature respires more rapidly than that of the same moisture content at lower out-door temperatures. Unsoundness of wheat caused by the freezing of unripe plants increases respiration. This is attributed to the accumulation of glucose in the frosted grains. Increased temperature increases the respiration up to 55° C. When seeds are stored in closed chambers and the respiration taken by 4-day periods, the rate is highest for the first period and diminishes materially in successive periods as the carbon dioxide content rises. The respiration is also reduced in an oxygen free atmosphere, the ratio to that occurring in a normal atmosphere being about 1:2.5.

Many will think the author's evidence for their viscosity conception of limited respiration is insufficient. They will also question whether the amount of glucose present limits respiration when low moisture has already run respiration to so low an ebb.—Wm. Crocker.

Relation of host and parasite among fungi.—An excellent service has been rendered by Reed[19] in bringing together the extensive and scattered data regarding the susceptibility of more or less related hosts to physiological strains

[18] Bailey, C. H., and Gurjar, A. M., Respiration of stored wheat. Jour. Agric. Research 12:685–713. 1918.

[19] Reed, George M., Physiological specialization of parasitic fungi. Mem. Brooklyn Bot. Gard. 1:348–409. 1918.

among various fungi. Some 68 species of fungi, the majority of them belonging to the Uredinales, have been reported to show such specialization. The first known and best studied species is *Puccinia graminis*, producing the destructive stem rust of wheat and of other cereals and grasses. A few species having a wide range of hosts, like *P. subnitens*, appear not to be specialized. The citation of literature includes 174 titles, supplied by 67 writers, indicating the prominence which this line of investigation has attained within the last few years. ERIKSSON's studies on the specialization of the grain rusts, reported in 1894, introduced the subject, but the fixed and unchanging character of physiological strains has first been shown definitely in the present paper, since being confirmed by STAKMAN and others.[20]

It is pointed out that so far the data do not indicate that bridging species are capable of altering the physiological nature of the parasite so as to enable it to extend the range of its natural hosts, as has heretofore been assumed. In fact, it appears that among fungous parasites there are definite strains or races not distinguishable morphologically, but only by their physiological behavior in infecting certain hosts, and that these strains retain the same characters through all the metamorphoses of the fungus, and when tested by use of any kind of reproductive body that the particular species produces. The specialization of the same fungus in widely separated regions may possibly be different, but the data are scanty. The relation of physiological specialization to morphological variation is barely mentioned. The whole subject of specialization is one of great scientific and economic interest, making the present admirable summary particularly timely.—J. C. ARTHUR.

Heath and grassland.—Continuing the investigations already noted[21] of certain English heaths and grassland, FARROW[22] has accumulated more data upon the effects of a rabbit population upon vegetation retrogression. It is demonstrated that the presence of rabbits alone is sufficient at times to change a pine forest through *Calluna* heath and *Carex arenaria* associations to a dwarf grass or a *Cladonia* heath. Experiments with irrigation and with the application of manure tend to show that both sterile soil and lack of soil moisture are factors in limiting the rate of growth and the luxuriance of the vegetation. This increased growth with improved conditions results in a decrease in the number of species in the area, since the more rapid growth of certain plants, like *Agrostis vulgaris*, smothered less vigorous ones, such as *Festuca ovina*.

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[20] STAKMAN, E. C., PARKER, J. H., and PIEMEISEL, F. J., Can biologic forms of stem rust on wheat change rapidly enough to interfere with breeding for rust resistance? Jour. Agric. Res. 14:111–123. *pls. 13–17*. 1918.

[21] BOT. GAZ. 64:263. 1917.

[22] FARROW, E. P., On the ecology of the vegetation of Breckland. III. General effects of rabbits on the vegetation. IV. Experiments mainly relating to the available water supply. V. Observations relating to competition between plants. Jour. Ecology 5:1–18, 104–112, 155–172. 1917.

Evidence is also presented that such plants as *Pteris aquilina* and *Pinus* often succeed in competition owing to their dead foliage excluding the light from their competitors, causing etiolation and decay.

In a more recent paper FARROW[23] has examined the retrogression begun by rabbits and continued by sand blasts. This retrogression shows exactly the reverse order of the succession inaugurated by irrigation, being particularly noticeable in the *Agrostis vulgaris* giving place to *Festuca ovina* wherever the sand blast became intensive. Once begun, bare areas tend to increase, the sand assisting in destroying the vegetation both by direct attack and by removing the substratum, leaving clumps of grass upon the tops of small hummocks which are being constantly undermined. With the checking of wind erosion in such bare areas *Polytrichum* and *Cladonia* become agents of stabilization and revegetation.—GEO. D. FULLER.

Photosynthesis.—OSTERHOUT and HAAS[24] summarize as follows a piece of work on the dynamics of photosynthesis. "*Ulva* which has been kept in the dark begins photosynthesis as soon as it is exposed to sunlight. The rate of photosynthesis steadily increases until a constant speed is attained. This may be explained by assuming that sunlight decomposes a substance whose products catalyze photosynthesis or enter directly into the reaction. Quantitative theories are developed to account for the facts." The rate of photosynthesis was determined by the rate at which a portion of *Ulva* rendered sea water basic to phenolphthalein. Since the dissociation of carbonic acid is very slight, change of reaction is a very crude way of measuring the amount present. There is also the possibility of other exchanges of more strongly dissociating materials that could modify the reaction of the water. In the face of excellent and very accurate methods for the quantitative determination of carbon dioxide it seems hardly justifiable to use this questionable method for a study of either respiration or photosynthesis. It is also doubtful whether sufficient regard has been given to other possible limiting factors of the rate of photosynthesis in these experiments. If, in spite of the defects of experimentation, the general conclusion proves true, it is a contribution of great significance and aids in confirming WILLSTÄTTER's view that the presence of a catalyzer is a common internal limiting factor to the rate of photosynthesis.—WM. CROCKER.

Organic plant poisons.—BRENCHLEY[25] finds hydrocyanic acid very toxic to pea and barley seedlings in water cultures. Hydrocyanic acid in concentrations of 1 part to 100,000 proved rather quickly fatal for peas and somewhat

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[23] FARROW, E. P., On the ecology of the vegetation of Breckland. V. Characteristic bare areas and sand hummocks. Jour. Ecology 6:144–152. 1918.

[24] OSTERHOUT, W. J. V., and HAAS, A. R. C., Dynamical aspects of photosynthesis. Proc. Nat. Acad. Sci. 4:85–91. 1918.

[25] BRENCHLEY, WINIFRED E., Organic plant poisons. I. Hydrocyanic acid. Ann. Botany 31:447–456. 1917.

less toxic for barley. Dilutions as great as 1 part to 4,000,000 to 10,000,000 proved somewhat toxic. Hydrocyanic acid showed no stimulation and the cyanogen radicle is the toxic agent.

BRENCHLEY[26] has also studied the effect of various phenols (phenol o-cresol, m-cresol, p-cresol, resorcinol, pyrocatechol, pyrogallol, phloroglucin, orcinol) upon the growth (as indicated by increased dry weight) of barley and peas in water cultures. The purpose was to learn the direct effects of these phenols on the plants, so that it could be considered in using the phenols as partial soil sterilizers. The following concentrations were used: M/100, M/100×1/5, M/100×1/5², and M/100×1/6². The general physiological effect was the same for all the phenols, but the concentration at which these effects showed varied considerably with the different members. The highest concentration was quickly fatal with all the phenols, and the next to highest concentration with o-cresol, pyrocatechol, and pyrogallol, but there was a slight recovery in the others. The lowest concentration showed no injury in any. None of the solutions showed any stimulus effect in any concentrations.—WM. CROCKER.

Regeneration in Phegopteris.—Miss BROWN[27] has recorded the results of some experiments on regeneration in *Phegopteris polypodioides*. Near the base of the petiole of a detached leaf regeneration took place in contact with sand moistened with Knop's solution in moist air. A prothallium-like growth appeared, and from this were developed rhizoids, structures intermediate between leaves and prothallia, and true leaves. The possible determining factors are enumerated, and among them the separation of the leaf from the parent body was evidently necessary; at least it seems evident that "some phase of nutrition must be an important factor in regeneration, if not the most important factor."—J. M. C.

Selaginella.—VAN ESELTINE[28] has begun a series of contributions dealing with the American species of *Selaginella* allied to *S. rupestris*. The group is in need of critical revision, and the results will be of interest to the morphologist as well as the taxonomist. The first paper deals with the representatives of the group occurring in the Gulf Coastal Plain and the territory immediately adjacent to the northeast. In this region 8 such species are recognized, 2 of which are described as new, and an additional one was described by the same author recently. The numerous drawings and photographic plates supplement well the full descriptions.—J. M. C.

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[26] BRENCHLEY, WINIFRED E., Organic plant poisons. II. Phenols. Ann. Botany **32**:259–278. 1918.

[27] BROWN, ELIZABETH W., Regeneration in *Phegopteris polypodioides*. Bull. Torr. Bot. Club **45**:391–397. *figs. 3.* 1918.

[28] VAN ESELTINE, G. P., The allies of *Selaginella rupestris* in the southeastern United States. Contrib. U.S. Nat. Herb. **20**:159–172. *pls. 15–22. figs. 63–70.* 1918.

Vegetation of a glacial plunge basin.—In certain rock basins of glacial origin near Syracuse, New York, low soil and air temperatures prevail throughout the year, the difference between the rim and bottom of the depressions often amounting to 30° F. These temperature depressions have been shown by PETRY[29] to be the controlling factors in the development of plant associations characterized by distinctly northern species, such as *Cornus canadensis*, *Pyrola asarifolia*, *Coptis trifolia*, and *Ribes lacustre*, whose local distribution coincide exactly with areas of low soil and air temperature.—GEO. D. FULLER.

Geotropism and phototropism.—VAN AMEIJDEN[30] finds that neither geo-perception nor photo-perception or reaction occurs in the seedlings of *Avena sativa* or *Sinapis alba* in complete absence of oxygen. Contrary to CORRENS and KENKEL, he finds that, on complete or partial withdrawal of oxygen, the reaction of seedlings to a geotropic stimulus does not differ from their reaction to a phototropic stimulus.—WM. CROCKER.

Rusts of Costa Rica.—ARTHUR[31] has studied the rusts of Costa Rica based chiefly upon collections made by HOLWAY, and this first presentation of Costa Rican rusts includes 118 species, 22 of which are described as new, and 12 others are new to North America. The indications are that the rust flora of Costa Rica will be found to be of exceptional richness and importance.—J. M. C.

Aquilegia.—PAYSON[32] has published a revision of the North American species of *Aquilegia*. In addition to the keys, descriptions, and discussions, there is an unusually full list of stations. He recognizes 25 species, 3 of which are described as new, and also 9 subspecies or varieties, 2 of which are new.—J. M. C.

New African plants.—MOORE,[33] in connection with his studies of African Compositae, has described a new genera (*Emiliella*) of the Senecionidae and 8 new species of *Senecio*.—J. M. C.

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[29] PETRY, LOREN C., Studies of the vegetation of New York State. II. The vegetation of a glacial plunge basin and its relation to temperature. Bull. Torr. Bot. Club 45:203–210. 1918.

[30] VAN AMEIJDEN, U. P., Geotropism and phototropism in the absence of free oxygen. Recueil Trav. Bot. Neerl. 14:149–218. *pls. 15–19. fig. 1.* 1917.

[31] ARTHUR, J. C., Uredinales of Costa Rica based on collection by E. W. D. HOLWAY. Mycologia 10:111–154. 1918.

[32] PAYSON, EDWIN BLAKE, The North American species of *Aquilegia*. Contrib. U.S. Nat. Herb. 20:133–157. *pls. 8–14.* 1918.

[33] MOORE, SPENCER LEM., Alabastra diversa. Part XXIX. Jour. Botany 56: 225–233. 1918.

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RELATION OF MINIMUM MOISTURE CONTENT OF SUBSOIL OF PRAIRIES TO HYGROSCOPIC COEFFICIENT[1]

F. J. Alway, G. R. McDole, and R. S. Trumbull

Introduction

It has long been recognized that the maxima and minima percentages of water found in well drained soils in the field are in general roughly dependent upon the relative fineness of texture, but very few data have been published in such form as to permit of any attempt to compute the actual relations which these extremes bear to the physical constants. In a previous paper (3) we have reported laboratory experiments and field observations showing that when loams, after rains sufficiently heavy to moisten them thoroughly, are protected from losses by evaporation and transpiration, they lose water by downward movement until the ratio of moisture content to hygroscopic coefficient reaches a value between 1.8 and about 2.5; while with coarse sands the ratio is as high as 6.0 or 7.0; and fine sands occupy an intermediate position, the ratio rising with a decrease in hygroscopicity.

While the maxima under field conditions are easily ascertainable anywhere, it being necessary only to await heavy rains or to irrigate a small area, the corresponding minima are developed only when a very scanty rainfall or a prolonged absence of precipitation is

[1] The work reported in this paper was carried out in 1907–1913, while the authors were members of the staff of the Nebraska Agricultural Experiment Station.

accompanied by weather conditions which stimulate both evaporation and transpiration, and so favor a reduction of the soil moisture content. These favoring conditions are high temperature, low atmospheric humidity, high wind velocity, and a high degree of insolation. Even in a semi-arid region several years may pass without the concurrence of the necessary conditions, while in humid regions such intervals are of still greater length.

The results of the greenhouse experiments of BRIGGS and SHANTZ (6) would suggest that after periods of extreme drought the prairie subsoil might be expected to show a moisture content which either approximated the so-called wilting coefficient (1.47 times the hygroscopic coefficient) or which was somewhat lower than the former value but bore no distinct relation to the latter. In the opinion of these authors the wilting coefficient "practically marks the cessation of growth," and after this point has been reached the soils continue to lose water through the tissues of the plants, even after they are dead, the final moisture content of the soil being as low as though the soil and air had been in direct contact. However, pot experiments of any kind, and especially those employing shallow vessels, appear ill adapted to answer the question as to how dry a particular soil may become under field conditions. Accordingly the data obtained in the field at such times as when the weather conditions have been favorable to an extreme reduction of the subsoil moisture should prove of especial interest, provided they are accompanied by determinations of the hygroscopic coefficient or wilting coefficient of the soils.

In regions of winter rains and summer droughts, such as California, one may safely count upon the continuance of hot rainless weather with clear skies for many weeks after the dry season has once set in, but in those with summer rains one never knows when to make preparations for studies that are dependent upon extreme brought conditions, and it may happen that, after all arrangements have been completed, several years may pass before weather conditions favoradle for the work occur. For this reason data on the soil moisture content during unusual droughts in humid regions are most apt to be secured in the course of some less specialized investigation.

Location of prairies sampled

The fields from which we secured the following data form two groups, one in the southwestern corner of Nebraska adjacent to the towns of McCook, Wauneta, Imperial, and Madrid; and the other close to the Nebraska Experiment Station at Lincoln. As all the former were at a distance of 200 miles or more from the experiment station, the sampling of them was feasible only at long intervals, and many of the data from these were secured incidental to the collection of samples for chemical studies. The western group of fields is beyond question well within the semi-arid region, while those at Lincoln lie almost as far to the west as the strictly humid climate extends on the American prairies. Some may consider that even Lincoln falls within the eastern limit of the great semi-arid region, but the composition of the soil (4, p. 414), the growth of vegetation and its agricultural history, as well as the moisture conditions of the subsoil distinguish it from the drier country west of Holdrege. All the factors which determine the difference in climate alter so gradually from east to west that it is impossible to place any definite line of demarcation between the humid and the semi-arid regions, the most that we are justified in assuming being that for every advance of a few miles to the westward of Hastings there is a nearer approach to strictly semi-arid conditions. At Hastings we appear to be still within the humid region, while at Holdrege, 50 miles farther west, most of the characteristics of semi-arid regions are discernible. Also the distribution of carbonates in the subsoil indicates that the district between Hastings and Holdrege is the region of most rapid transition (4, p. 414).

Favorable weather conditions

The weather of the period covering our work proved extremely favorable for the development of dry subsoils in both localities. At Lincoln it included the driest two-year period (1911–1912) of the past 20 years, 1897 to 1916, although in two years, 1895 and 1901, there had been a lower annual precipitation than in either of these (table I). Accordingly the soil moisture conditions we found there may be considered to include those representing the effects of extreme drought.

In southwestern Nebraska our work was begun in seasons, 1907 and 1908, forming the conclusion of a series of wet years. This was followed by a prolonged dry period, reaching its climax in

TABLE I

RELATION OF ANNUAL PRECIPITATION AT LINCOLN, YEAR BY YEAR, TO NORMAL (=100), 27.51 INCHES, SHOWING RELATIVE DRYNESS OF PERIOD OF OBSERVATION (1906 TO 1912).

Year	Percentage	Year	Percentage	Year	Percentage	Year	Percentage
1895....	60	1901....	80	1907....	99	1913....	95
1896....	138	1902....	150	1908....	130	1914....	145
1897....	93	1903....	126	1909....	126	1915....	128
1898....	102	1904....	101	1910....	114	1916....	80
1899....	82	1905....	129	1911....	89	.....	.....
1900....	123	1906....	124	1912....	81	.....	.....

1910–1911, the precipitation in 1910 being the lowest recorded since observations were begun at North Platte 42 years ago (table II). By 1911 the subsoil moisture had probably been reduced to as

TABLE II

ANNUAL PRECIPITATION IN INCHES AT STATIONS IN SOUTHWESTERN NEBRASKA

Length of record in years*	McCook 16	Wauneta 27	Imperial 26	H. O. Ranch 11	North Platte 42
Normal	19.71	18.70	20.79	17.80	18.88
1905	33.97	32.24	33.05	.....	26.81
1906	20.59	22.82	26.23	20.14	27.99
1907	19.32	20.18	16.76	12.02	19.61
1908	18.08	24.77	26.27	21.01	19.96
1909	22.54	18.46	20.03	16.89	22.41
1910	9.34	13.82	11.77	7.62	10.70
1911	12.15	18.82	17.37	12.76	17.43
1912	14.69	20.00	24.58	20.74	18.69
1913	18.26	16.05	16.60	14.99	19.10
1914	18.24	17.26	16.94	19.42	15.79
1915	30.95	27.04	37.14	35.84	32.70
1916	15.35	14.95	19.33	14.60	12.96
Maximum	33.97	32.24	37.14	35.84	32.70
Minimum	9.34	13.82	11.77	7.62	10.70

* To end of 1916.

low a point as is ever experienced in southwestern Nebraska. The climate of the Nebraska portion of the Transition Region, including both groups of fields, has been discussed in some detail in a previous paper dealing with the composition of its loess soils (2).

Experimental methods

In taking the samples we used augers provided with extensions, commonly employing two sizes, one 1.5 inches in diameter with which to take the sample, and another 2.0 inches in diameter to enlarge and clean out the hole preparatory to sampling the next lower section. In many of the borings in western Nebraska the subsoil in part or in all the levels sampled was too dry to be removable by the ordinary auger, sliding off the bit as this was being withdrawn. In such cases we employed a Tinsley "auger with casing" (11), the sleeve on this retaining the soil loosened by the bit. Except where otherwise indicated, the samples were composites from 3 borings 10–20 yards apart. Composites were made from the first 3 borings only where it could be seen from the behavior of the soil toward the auger that the general moisture conditions in all 3 were similar, but not necessarily identical.

Extremes under semi-arid conditions

AFTER A PROLONGED DROUGHT.—As already stated, 1910 proved the driest year in southwestern Nebraska since observations were begun, the precipitation amounting to scarcely half the normal (table III). The autumn of this year and the following winter and spring were practically without snow or rain until April, the total precipitation at McCook from the end of August 1910 to the first of the following April amounting to only 1.60 inches (table IV), and this fell in such small amounts as to influence the soil moisture content through only a negligible distance. At Imperial and Wauneta the weather was not quite so dry, but the difference was not sufficient to cause an appreciable difference in the moisture content of the subsoil.

The samplings made in fields near McCook and Wauneta near the end of October 1910 showed such low ratios of moisture content to hygroscopic coefficient (table V) that some undiscovered source of error was suspected, and for this reason 6 weeks later we resampled two of them, A and B at McCook, and took sets from two additional fields. These confirmed the correctness of the extremely low ratios. The concordance of the moisture content with the hygroscopic coefficient was very striking, as though the

plant roots, while not recognizing the wilting coefficient, practically ceased to withdraw water as soon as the hygroscopic coefficient had been reached. There was little difference in moistness between

TABLE III

MONTHLY PRECIPITATION IN INCHES AT McCOOK, WAUNETA, IMPERIAL, AND THE H. O. RANCH, SHOWING DRYNESS OF SEASONS

MONTH	NORMAL			1910			
	McCook	Wauneta	Imperial	McCook	Wauneta	Imperial	H. O. Ranch
January	0.21	0.26	0.44	0.0	0.0	0.40	0.35
February	0.62	0.69	0.69	0.0	0.0	0.10	0.06
March	0.73	1.03	1.33	0.0	0.0	0.38	0.36
April	1.89	2.04	2.27	0.76	0.82	0.71	0.60
May	2.82	2.54	2.82	2.77	2.00	1.98	2.25
June	3.29	3.34	3.34	1.12	3.44	2.51	1.24
July	3.09	2.47	2.91	0.70	0.77	0.72	0.34
August	2.55	2.74	2.73	2.93	2.64	2.82	0.97
September	1.72	1.35	1.34	0.72	3.20	1.58	1.28
October	1.03	1.13	1.10	0.17	0.0	T	0.0
November	0.56	0.39	0.50	0.0	0.10	T	0.03
December	0.57	0.57	0.72	0.17	0.85	0.57	0.14
Annual	19.08	18.55	20.19	9.34	13.82	11.77	7.62

MONTH	1911				1912			
	McCook	Wauneta	Imperial	H. O. Ranch	McCook	Wauneta	Imperial	H. O. Ranch
January	0.05	0.10	0.42	0.15	0.0	0.20	0.52	0.15
February	0.47	0.70	0.37	0.31	0.24	1.51	1.08	1.00
March	0.12	0.50	0.22	0.03	1.50	2.35	3.61	2.70
April	1.72	3.45	2.55	1.94	2.01	2.82	2.85	1.74
May	1.25	1.75	2.19	1.29	0.0	0.95	1.41	1.53
June	0.66	1.35	1.29	0.92	2.77	1.89	1.82	1.80
July	0.84	1.30	1.10	0.84	2.29	3.26	5.09	5.16
August	4.34	3.07	3.45	1.97	2.11	2.78	4.28	2.49
September	0.59	1.80	1.44	0.86	2.13	2.61	2.01	2.41
October	0.96	3.30	2.92	2.85	1.09	1.43	1.55	1.45
November	0.05	0.0	0.10	0.05	0.50	0.20	0.15	0.0
December	1.10	1.50	1.32	1.55	T	0.0	0.21	0.05
Annual	12.15	18.82	17.37	12.76	14.69	20.00	24.58	20.44

the surface foot and the succeeding 2 or 3 ft., and even the deeper subsoil was but little if at all moister. At no level and in none of the fields was there any growth water, the moisture content being below the computed wilting coefficient, which corresponds to a ratio of 1.47, or approximately 1.5 (6).

TABLE IV

DAILY PRECIPITATION AT MCCOOK FROM SEPTEMBER 1, 1910, TO JUNE 30, 1912

	1910				1911												1912					
	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	March	April	May	June
1					0.05														T			
2		0.17										0.57							0.80			
3								0.89		0.36												
4								0.09			0.07	0.57	0.12									
5	0.15			0.10							0.07	0.47			T							
6												0.22		0.06								0.15
7												0.20										0.05
8																		T				0.95
9											0.27											0.33
10										0.12												
11												0.31			T							0.47
12												0.77						0.08	0.60			0.10
13				0.07							0.07											
14																						
15														0.41								
16						0.25				0.18				0.49	0.05							0.38
17						0.08		0.18														0.27
18												0.43				T						0.07
19											0.09											
20												0.73	0.19			0.95				0.55		
21	0.23								1.10										0.10			
22											0.27											
23	0.34																			0.08		
24																						
25								0.42								0.15		0.11				
26							0.12					0.07										
27									0.15													
28						0.14												0.05		1.38		
29													0.28									
30								0.14														
31																						
Total.	0.72	0.17	0.0	0.17	0.05	0.47	0.12	1.72	1.25	0.66	0.84	4.34	0.59	0.96	0.05	1.10	0.0	0.24	1.50	2.01	0.0	2.77

TABLE V

MOISTURE CONDITIONS AT McCOOK AND WAUNETA, AUTUMN 1910

A: McCOOK

DEPTH	OCTOBER 24		OCT. 26	OCT. 27	DECEMBER 9		DECEMBER 10	
Foot	Field A	Field B	Field C	Field D	Field A	Field B	Field D	Field E
	HYGROSCOPIC COEFFICIENTS							
1	9.2	9.5	10.2	8.8	9.1	10.0	9.3	10.6
2	10.7	11.4	11.3	10.9	10.4	10.1	10.9	9.9
3	10.4	9.1	10.2	10.7	10.2	9.1	10.7	8.2
4	10.1	8.6	8.4	10.8	9.3	7.8	9.6	8.1
5	9.4	8.5	8.6	.....	8.7	8.2	9.7	7.9
6	8.7	8.4	9.0	8.5	8.2	8.2	11.7	7.5
Average	9.8	9.1	9.6	9.6	9.3	8.9	10.3	8.7
	RATIOS							
1	0.8	0.8	0.8	0.8	1.1	0.9	0.9	0.9
2	0.9	0.8	0.9	0.8	1.0	0.8	0.8	0.9
3	0.9	0.8	0.8	0.8	1.0	0.9	0.8	0.9
4	0.9	0.9	0.9	0.9	1.0	1.0	1.0	1.0
5	0.9	1.1	1.0	.....	1.1	1.0	1.0	1.0
6	1.2	1.1	0.9	1.0	1.1	1.1	0.9	1.1
Average	0.9	0.9	0.8	0.9	1.0	0.9	0.9	1.0

B: Wauneta

DEPTH	OCTOBER 31	NOVEMBER 1		NOVEMBER 2	NOVEMBER 3
Foot	Field A	Field B	Field C	Field D	Field E
	HYGROSCOPIC COEFFICIENTS				
1	8.8	9.9	9.5	8.5	8.1
2	9.5	10.1	9.4	9.3	9.5
3	8.7	10.6	7.7	9.7	9.7
4	8.9	10.7	8.6	9.5	8.7
5	10.0	9.8	7.6	8.7	7.3
6	10.3	8.7	7.6	8.0	6.4
Average	9.4	10.0	8.4	8.9	8.3
	RATIOS				
1	0.9	0.8	0.9	1.0	0.9
2	0.8	1.0	0.9	0.8	1.0
3	0.9	0.9	1.0	0.8	0.9
4	1.0	1.0	0.9	0.8	1.0
5	1.0	0.9	1.0	0.9	1.1
6	1.1	1.2	1.1	1.1	1.3
Average	0.9	1.0	1.0	0.9	1.0

Some 4 months later 3 of the fields at McCook and Wauneta were sampled again and one added at the latter place. The

TABLE VI

MOISTURE CONDITIONS IN WESTERN NEBRASKA IN LATE SPRING OF 1911, FOLLOWING VERY DRY AUTUMN, WINTER, AND EARLY SPRING

DEPTH	McCOOK		WAUNETA		IMPERIAL				
	Mar. 24	Mar. 25	April 4		April 1	April 3	April 12	April 12	April 14
Foot	Field B	Field E	Field E	Field F	Field A	Field B	Field C	Field D	Field E
	HYGROSCOPIC COEFFCIENTS								
1	9.6	8.7	8.7	9.0	6.4	8.3	3.0	2.0	3.6
2	10.5	10.1	9.2	9.6	7.9	10.7	2.6	2.2	4.6
3	9.1	9.6	9.1	10.9	8.1	9.0	2.4	2.0	5.9
4	8.3	9.0	9.2	10.0	6.8	7.8	4.1	2.0	4.8
5	8.1	8.6	9.6	8.8	4.2	6.8	4.5	2.1	4.4
6	8.1	9.0	8.3	7.7	3.9	6.8	4.5	2.0	3.6
7	8.1	7.9	6.6	6.7	4.0	.....	5.1	1.9	4.2
8	8.3	8.6	5.7	7.3	5.3	.....	4.3	2.5	8.4
9	8.1	8.4	4.9	7.0	6.0	.....	3.5	2.0	5.4
10	8.1	8.4	4.4	7.0	.....	.....	.....	.....	.....
11	8.1	8.6	4.0	7.9	.....	.....	.....	.....	.....
12	8.1	8.6	3.7	7.4	.....	.....	.....	.....	.....
13	.....	.....	4.3	6.6	.....	.....	.....	.....	.....
14	.....	.....	4.2	6.5	.....	.....	.....	.....	.....
15	.....	.....	5.1	6.4	.....	.....	.....	.....	.....
Average	8.9	9.2	9.0	9.3	6.2	8.2	3.5	2.1	4.5
	RATIOS								
1	0.8	0.8	1.0	1.0	0.8	0.9	1.2	1.1	0.7
2	0.8	0.9	0.8	0.9	0.9	0.9	1.5	1.1	0.9
3	0.9	0.9	0.9	0.8	0.9	0.8	1.2	1.1	0.9
4	0.9	0.9	1.1	0.8	0.9	0.8	1.1	1.2	0.9
5	1.0	1.0	1.3	0.9	1.0	1.0	1.2	1.0	0.9
6	1.1	0.9	1.2	1.0	1.0	1.0	1.0	1.1	1.0
7	1.1	1.1	1.5	1.1	1.1	.....	1.1	1.4	1.4
8	1.1	1.1	1.5	1.0	1.2	.....	1.2	1.2	1.2
9	1.1	1.1	1.5	1.1	1.3	.....	1.2	1.4	1.3
10	1.2	1.1	1.5	1.1	.....	.....	.....	.....	.....
11	1.2	1.0	1.6	1.0	.....	.....	.....	.....	.....
12	1.2	1.1	1.8	1.1	.....	.....	.....	.....	.....
13	.....	.....	1.6	1.2	.....	.....	.....	.....	.....
14	.....	.....	1.7	1.1	.....	.....	.....	.....	.....
15	.....	.....	1.7	1.2	.....	.....	.....	.....	.....
Average 1–6	0.9	0.9	1.0	0.9	0.9	0.9	1.2	1.1	0.9

sampling was carried down to the twelfth or fifteenth foot on this occasion (table VI). The subsoil was not found appreciably drier than when first sampled. As 4 or 5 months of rainless weather had

intervened, it would appear that the loss of moisture by transpiration during the winter must have been very slight.

It is of interest that in the spring of 1911 the subsoil at depths of 7–15 ft. in field E at Wauneta was found quite moist, showing an average ratio of 1.6. The explanation of this will be discussed in a later paragraph.

On the last occasion samples were taken from near Imperial also. While the soil and subsoil in all of the fields at McCook as deep as sampled, and in all those at Wauneta except in the lower levels of E and F, were derived from the loess, and showed hygroscopic coefficients between 7.5 and 11.0, all the soils and subsoils at Imperial were residual in origin with hygroscopic coefficients varying all the way from 2.0 to 10.7. At Imperial, in contrast to McCook and Wauneta, we sampled some fine sandy loams as well as the more numerous fine textured soils, none of the latter, however, being of loessial origin. Comparing the ratios it will be seen that the prairies with the finer soil, A, B, and E, were as dry in the first 6 ft. as those at Wauneta and McCook, and that the one of the two with sandy soil and subsoil, C and D, was but slightly more moist. Below the sixth foot they were distinctly moister, but in none of them was the ratio much above 1.0. The reduction of the moisture content to the hygroscopic coefficient was general and was independent of the relative hygroscopicity.

After a wet winter following a prolonged drought.—Until the spring of 1912 no more sampling was done at McCook, Wauneta, or Imperial. The weather of the intervening months had been unfavorable for any marked increase in the moisture content of the deeper subsoil, although very favorable for moistening the surface foot. April and May of 1911 had a rainfall somewhat below normal, June and July were very dry, while during August and the first few days of September considerably more rain than usual fell. The rest of September was dry, but the precipitation of October was 3 times the normal and of such a character that there was little chance for run-off; as vegetation had become dormant the loss by transpiration must have been slight. November was very dry, but the precipitation of December was twice the normal, a heavy rain on December 20 further moistening the

surface soil. January and February together had a precipitation somewhat below normal at McCook, but above at both Wauneta and Imperial, while March at all 3 places had a precipitation 2 or 3 times the normal. April was rainless until the 20th, between which date and the 28th from 2 to 3 inches of rain fell.

Sampling was carried out at McCook on May 7 and 8, no rain having fallen since April 28; at Imperial on May 11, 13, and 14, 0.45 inch having fallen there in 4 light showers; and at Wauneta on May 16 and 17. At the last place the only rain since April 28 had been one of 0.10 inch on May 10. Thus conditions had been ideal for the downward movement of the water into the subsoil, while at each place an interval of 8–19 days had elapsed between the last good rain and the date of sampling.

The generally favorable weather of autumn, winter, and spring was evidenced by the circumstance that in the early spring the outlook appeared unusually promising for the farmers. Wheat had come through the winter in fine condition and preparations were being made for seeding a large acreage to spring grains, the prospects being considered so favorable that local merchants were willing to furnish seed grain in return for a reasonable share of the crop. Conditions appeared ideal for a study of the degree to which the ratio in the surface soil had to be raised before water could pass downward into the deeper portions of the subsoil, where during the previous year the moisture had been reduced to the hygroscopic coefficient or even slightly below.

In the fields with heavier soil we found that the moisture content had been distinctly affected at McCook (table VII) to only 2 ft., at Wauneta in the one field to 3 ft., in the other to 4 ft. or more, and in the only one sampled at Imperial to 5 ft.

In normal seasons.—That the low ratios prevailing throughout the subsoil of the prairies after severe droughts, as illustrated in the preceding tables, are not entirely absent even in favorable seasons, may be seen from table VIII reporting conditions at the H. O. Ranch. There, as at McCook, Wauneta, and Imperial, after periods of drought the ratio was found not far from 1.0 at all depths, while under more favorable conditions, as in July 1908, the low ratio was still to be found at some level within the first 6 ft.

While after protracted droughts and probably also after extremely wet periods the moisture conditions in the subsoil are quite uniform, they vary much from place to place under more normal weather conditions, as illustrated by table IX.

TABLE VII

MOISTURE CONDITIONS IN WESTERN NEBRASKA IN MAY 1912, AFTER WET WINTER AND SPRING

DEPTH	MCCOOK			WAUNETA		IMPERIAL					
Foot	May 7	May 7	May 8	May 16	May 17	May 11			May 13		May 14
	Field A	Field B	Field E	Field B	Field C	Field E	Field G	Field H	Field I	Field J	Field K
	HYGROSCOPIC COEFFICIENTS										
1	8.4	9.6	10.1	9.2	7.5	8.2	1.6	2.6	7.1	5.8	3.2
2	9.6	10.3	10.1	10.4	9.1	10.2	2.6	3.7	7.5	6.3	3.2
3	8.1	8.3	.....	10.0	9.0	8.9	1.9	3.5	9.7	7.1	5.4
4	8.7	7.5	.....	9.6	9.5	5.3	1.5	3.5	9.0	5.1	3.7
5	8.4	7.8	7.3	8.4	8.4	4.7	1.3	1.6	.....	.....	3.4
6	7.2	7.6	.....	7.6	6.8	4.9	1.3	1.3	.....	.....	3.0
Average	8.4	8.5	.....	9.2	8.4	7.0	1.7	2.7	.....	.....	3.7
	RATIOS										
1	2.6	2.1	2.1	2.0	2.3	2.0	2.5	2.9	2.4	2.1	2.4
2	1.4	1.4	1.3	1.9	2.2	1.7	2.1	2.8	2.4	2.3	3.5
3	1.1	1.1	.....	1.4	1.6	1.3	4.2	2.7	1.5	1.3	2.0
4	1.0	1.1	.....	1.0	1.3	1.2	4.6	2.3	1.1	1.2	1.1
5	1.1	1.0	1.0	1.1	1.2	1.2	4.5	3.1	.....	.....	1.1
6	1.3	1.1	.....	1.2	1.3	1.0	4.7	3.4	.....	.....	1.2
Average	1.4	1.3	.....	1.4	1.6	1.4	3.8	2.9	.....	.....	1.9

Computations from data of Shantz and of Burr

The only data reported by other investigators that may be used for comparison with our own appear to be those secured by SHANTZ at Akron, Colorado, in 1909, and by BURR at North Platte, Nebraska, in 1912. While neither of these authors reports the hygroscopic coefficients of the soils, each gives the wilting coefficients for a representative set of samples, these having been computed from the determined moisture equivalents. From these data we have computed the hygroscopic coefficients by means of the Briggs-Shantz formula (6, p. 65): hyg. coef. = wilt. coef. $\times 0.68$.

For the period June 7–September 27, 1909, SHANTZ determined the moisture content twice a day in a grama-buffalo grass association, recording it in 6-inch sections to a depth of 3 ft. and in foot sections through the succeeding 3 ft. From his data we find that on August 7–8 and 10–11 the ratio of the moisture content to the

TABLE VIII

DATA FROM H. O. RANCH IN DIFFERENT YEARS, INCLUDING THE FAVORABLE SEASONS OF 1907 AND 1908

DEPTH	1907	1908		1910		1911	1913
Foot	Nov. 22	April 30	July 29	March 24	Sept. 21	April 21	July 13
	HYGROSCOPIC COEFFICIENTS						
1	7.7	8.5	8.0	8.7	8.8	8.9	8.4
2	10.3	9.8	9.7	9.7	11.5	11.1	
3	10.1	9.8	11.3	10.4	9.2	10.5	
4	7.7	8.3	7.7	8.7	7.9	8.6	8.6
5	7.1	6.9	6.4	7.9	7.5	8.5	
6	7.2	7.4	6.3	7.5	6.5	7.5	
7	6.4	.....	5.9	6.8	.....	7.2	
8	.....	.....	5.1	6.1	.....	6.8	6.4
9	8.3	.....	5.9	6.0	.....	7.7	
Average 1–6	8.3	8.4	8.2	8.8	8.6	9.2	7.6
	RATIOS						
1	1.9	1.2	2.1	2.1	1.2	0.7	0.9
2	1.1	1.1	2.0	1.2	0.8	0.8	
3	1.1	0.9	1.1	1.0	0.8	0.8	
4	1.3	1.0	1.4	1.0	0.9	0.9	0.9
5	1.5	1.1	1.6	1.0	0.9	1.0	
6	1.8	1.1	1.8	1.1	1.1	1.1	
7	1.8	.....	1.5	1.1	.....	1.1	
8	.....	.....	1.6	1.1	.....	1.2	1.2
9	.....	.....	1.3	1.1	.....	.....	
Average 1–6	1.5	1.1	1.7	1.2	0.9	1.0	1.0

hygroscopic coefficient in the 7–12 and 13–18 inch levels fell to approximately 1.0 (**10**, p. 35); while from July 22 to September 9 the ratio at the latter depth was almost continuously much below 1.5. From August 7 to 13 the sixth foot, and to a less extent the fifth, showed ratios close to 1.0. In each of the 4 months included in the study the rainfall was much above the normal for Akron, the excess varying from 25 to more than 100 per cent (8), and

averaging at least 50 per cent above the normal. There was no actual drought at any time during the season, but there were two rather dry periods, June 14–July 6 and July 11–25, in which light rains gave totals of 0.20 and 0.09 inch respectively.

When the subsoil at Akron, even in that unusually wet summer, had its moisture content reduced to such a low point, it is probable

TABLE IX

DATA FROM 6 INDIVIDUAL BORINGS ON THE H. O. RANCH, NOVEMBER 22, 1907, ILLUSTRATING VARIATIONS FROM BORING TO BORING

DEPTH	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	Average	Maximum	Minimum
Foot	HYGROSCOPIC COEFFICIENTS								
1	6.9	7.4	7.8	7.6	7.2	6.1	7.1	7.8	6.1
2	8.4	8.6	10.4	10.2	8.3	6.7	8.8	10.4	6.7
3	7.3	6.7	10.2	10.0	8.1	7.5	8.4	10.2	6.7
4	8.5	5.1	7.0	8.4	9.9	7.7	7.9	9.9	5.1
5	7.3	4.2	7.0	7.1	11.8	6.7	7.3	11.8	4.2
6	7.2	5.3	7.8	6.5	9.9	5.4	7.0	9.9	5.3
7	7.9	6.7	6.9	6.0	9.2	5.2	7.3	9.2	5.2
Average 1–7	7.6	6.5	8.1	8.0	9.2	6.5	7.6	9.2	6.3
	RATIOS								
1	1.6	1.7	1.8	1.9	1.7	2.0	1.8	2.0	1.6
2	1.3	1.1	1.2	1.1	1.2	1.5	1.2	1.5	1.1
3	1.3	1.1	1.1	1.1	1.2	1.1	1.2	1.3	1.1
4	1.2	1.1	1.5	1.1	1.1	1.2	1.2	1.5	1.1
5	1.2	1.3	1.7	1.3	1.4	1.2	1.3	1.7	1.2
6	1.3	1.4	1.7	2.0	1.4	1.3	1.4	2.0	1.3
7	1.3	1.7	1.9	1.8	1.2	1.4	1.5	1.9	1.2
Average 1–7	1.3	1.3	1.6	1.5	1.3	1.4	1.4	1.6	1.3

that in a really dry season the ratios would be found as low as those we encountered in southwestern Nebraska.

The root systems of the native plants were studied by SHANTZ, but the penetration of the grama and buffalo grasses he indicates (10) would not account for the removal of available moisture from below the first foot or two.

BURR (7) reports data from a prairie sampled in the spring, summer, and early autumn of 1912 (table X). In the spring high ratios were shown in the first 2–3 ft., but by the end of June the

ratios at all levels sampled had fallen to practically 1.0 and so remained through the remainder of the season, there being no evidence of further drying of the subsoil.

Thus the data of both SHANTZ and BURR confirm our findings regarding the stage of dryness to which the subsoil may be reduced in a dry season by the short grass vegetation, while those of the latter author agree also with our view that after the subsoil moisture content has been reduced to approximately the hygroscopic coefficient it suffers but little, if any, further lowering through a continuation of the drought conditions, and not with that of BRIGGS and SHANTZ that the subsoil continues to lose water through the plant tissues until it approaches an air-dry condition (6, p. 8).

TABLE X

RATIO OF MOISTURE CONTENT TO HYGROSCOPIC COEFFICIENT IN A PRAIRIE FIELD AT NORTH PLATTE IN 1912, COMPUTED FROM DATA OF BURR

Depth foot	Hygroscopic coefficient*	April			May		June		July		Aug.	Sept.
		18	22	29	11	25	10	29	25	26	22	12
1.....	6.8	2.5	3.3	3.3	3.1	2.0	1.4	0.8	1.0	1.8	0.8	1.5
2.....	6.8	1.9	2.2	3.2	2.6	2.2	1.3	0.8	0.9	0.9	0.8	0.9
3.....	6.8	1.2	1.2	1.7	2.3	2.0	1.6	0.9	0.9	0.9	0.8	0.9
4.....	6.8	1.1	1.2	1.2	1.4	1.5	1.4	0.9	1.0	1.1	0.9	0.9
5.....	7.5	1.1	1.1	1.2	1.2	1.2	1.3	0.8	1.0	1.1	0.8	0.8
6.....	7.5	1.1	1.2	1.2	1.0	1.1	1.2	0.7	0.9	1.1	0.8	0.9

* Computed from wilting coefficients of a representative set of samples (7).

Extremes in eastern Nebraska

The periods of extreme drought at Lincoln were not numerous, and usually when these came an examination with the soil auger showed that the moisture content of even the surface foot or two was well above the hygroscopic coefficient, and as it was only the minimum moisture content that we were seeking in these prairie fields we report data from only a few sets of samples. On only 3 occasions in the 6-year period (1906–1912) did we find in the surface 2–3 ft. the dry condition which indicates the approaching exhaustion of available moisture (table XI).

The first sampling, on August 23, 1909, had been preceded by a comparatively dry period of 42 days, during which only 1.57 inches of rain had fallen; none had fallen in the last 20 days, while the

weather had been unusually hot and windy. The time of the season was that at which the draft upon the subsoil moisture might be expected to show the most marked effect. In the soil of the first foot we found a ratio of 1.0 and in that of the next 3 ft., an average ratio of 1.4; but the sixth foot, with a ratio of 1.9, appeared to have lost but little of the moisture which it could retain against downward movement.

TABLE XI

MOISTURE CONDITIONS IN PRAIRIE FIELDS NEAR LINCOLN AFTER UNUSUALLY DRY PERIODS

AUGUST 23, 1909			AUGUST 3, 1911			JUNE 7, 1912		
Depth foot	Hygroscopic coefficient	Ratio	Depth foot	Hygroscopic coefficient	Ratio	Depth foot	Hygroscopic coefficient	Ratio
1.....	10.9	1.0	1.....	10.5	1.4	1.....	13.4	1.5
2.....	10.8	1.3	2–5...	13.1	1.3	2.....	15.2	1.3
3.....	11.5	1.5	6–8...	12.3	2.0	3.....	14.1	1.2
4.....	14.0	1.5	9.....	12.8	2.1	4.....	13.9	1.2
5.....	12.5	1.6	.....	.....	.....	5.....	12.5	1.5
6.....	11.8	1.9	.....	.....	.....	6.....	12.2	1.9

On the second occasion, August 3, 1911, a dry period of 65 days had just been ended by a rain of 0.84 inch. Compared with a normal precipitation of 9.0 inches for this period, only 2.68 inches of rain had fallen, and this in light showers, while both the mean temperature and the wind velocity had been somewhat above the normal. As the subsoil of the second to the fifth foot appeared uniformly dry it was combined into a single sample, the ratio proving to be 1.3, but in the sixth to ninth foot it was 2.0 to 2.1. The moister condition in the surface foot indicated in the table was due to a shower of the day before having moistened the immediate surface layers.

From the time of the preceding to the next and last sampling, June 7, 1912, the weather on the whole was very unfavorable to the accumulation of any moisture in the subsoil, and the spring of 1912 was exceptionally favorable to the exhaustion of whatever available water was within reach of the plant roots. The moisture conditions found were quite similar to those on the preceding occasion.

Thus the samplings, taken at times of drought when one might have expected almost the lowest moisture content in the subsoil

ever to be found in prairie fields at Lincoln, showed dry subsoil only within the first 5 ft., below this depth the ratios lying between extremes of 1.9 and 2.7, or in general between 2.0 and 2.4, the moisture retaining capacity of the subsoil. On only one occasion, and then only in the first foot, was the moisture content found reduced as low as the hygroscopic coefficient, and there it is to be attributed to the surface few inches of the foot section having been dried by evaporation to a point much below this value, with the result that the average for the whole foot section shows a low ratio.

In this connection it is of interest to know the ratios which normally prevail in the deeper subsoil of the eastern prairies. In April 1911, a field situated on a gentle slope and 50 ft. or more above ground water was sampled to a depth of 18 ft. (table XII). Below the fifth foot ratios ranging only between 2.1 to 2.4 were found.

TABLE XII

MOISTURE CONDITIONS IN PRAIRIE NEAR LINCOLN, APRIL 13, 1911, SHOWING NORMAL CONDITION OF DEEPER SUBSOIL

Depth foot	Hygroscopic coefficient	Ratio	Depth foot	Hygroscopic coefficient	Ratio	Depth foot	Hygroscopic coefficient	Ratio
1.....	11.8	2.5	7....	13.0	2.2	13....	12.3	2.2
2.....	15.3	2.0	8....	12.8	2.2	14....	12.0	2.4
3.....	14.3	1.8	9....	13.6	2.1	15....	12.3	2.3
4.....	14.2	1.8	10....	12.1	2.4	16....	10.3	2.3
5.....	13.6	1.9	11....	13.4	2.1	17....	9.8	2.3
6.....	13.1	2.1	12....	13.0	2.2	18....	10.9	2.2

Thus as near the surface as the sixth foot, when conditions were such as to develop the driest subsoil, the ratio was not much below that found in the deep subsoil under normal conditions. This failure of the natural vegetation of the prairies of eastern Nebraska to exhaust the free water of the deeper subsoil is in sharp contrast with the conditions found on the short-grass prairies of the southwestern part of the state, as previously described. That this moist condition is due to a difference in the conduct of the native plants and not to any peculiar properties of the humid subsoil is evident from the fact that in the alfalfa fields adjacent to the prairies kept under observation the ratios were quite commonly found reduced as low as 1.2 to 1.4 to a depth of 15 or 20 ft.,

or even more (table XIII). In an oak grove planted on the prairie some 30 years before and sampled on practically the same dates

TABLE XIII

MOISTURE CONDITIONS IN EASTERN NEBRASKA ALFALFA FIELD, ADJACENT TO PRAIRIE REPORTED IN TABLE XII, SHOWING FAILURE OF PRAIRIE VEGETATION TO REDUCE MOISTURE CONTENT OF DEEPER SUBSOIL NOT DUE TO ANY PECULIARITY OF SUBSOIL

APRIL 13, 1911			SEPTEMBER 12, 1912			
			Boring 1		Boring 2	
Depth foot	Hygroscopic coefficient	Ratio	Hygroscopic coefficient	Ratio	Hygroscopic coefficient	Ratio
1.....	11.6	2.4	13.2	1.4	12.9	1.3
2–6.....	13.4	1.7	13.7	1.1	13.5	1.1
7–12.....	11.0	1.5	12.4	1.1	12.1	1.1
13–18.....	8.5	1.5	10.5	1.1	10.8	1.1
19–21.....	11.5	1.4	11.8	1.2	12.1	1.1

as the prairie fields, the subsoil moisture was found to be affected to a greater depth than in the latter, the drying effect extending apparently to at least 15 ft. (table XIV).

TABLE XIV

MOISTURE CONDITIONS IN AN OAK GROVE NEAR LINCOLN

AUGUST 23, 1909			AUGUST 2, 1911			JULY 5, 1912		
Depth foot	Hygroscopic coefficient	Ratio	Depth foot	Hygroscopic coefficient	Ratio	Depth foot	Hygroscopic coefficient	Ratio
1.....	10.1	1.1	1.....	9.1	1.8	1....	10.0	0.7
2.....	11.7	1.3	2–9...	12.4	1.3	2....	11.8	1.7
3.....	14.2	1.3	10–14.	11.9	1.6	3....	14.2	1.4
4.....	14.1	1.2	15....	12.0	1.6	4....	14.1	1.2
5.....	13.9	1.2	.....	.....	.....	5....	14.0	1.2
6.....	13.4	1.2	.....	.....	.....	6....	13.5	1.2

Discussion

The moisture conditions in the deeper subsoil of the prairies are very dissimilar according to whether we deal with humid or with semi-arid fields. In the former at depths below 6 ft. the subsoil appears always moist, even after the severest drought, while in the latter the extreme dryness indicated by ratios of 1.0–1.2 is in general persistent in the deeper subsoil, extending to a depth of

12 ft. or more after prolonged droughts, and even in wetter seasons is commonly found in one or more foot levels within the first 6 ft. That the lack of dryness in the deeper subsoil of the humid prairies is not due to any peculiarity of the subsoil is evident from the observation that a fair stand of alfalfa may in the course of a few years reduce the moisture content almost to the hygroscopic coefficient to a depth of 20 ft. or more.

In our deep cylinder experiments (1) the exhaustion of free water was observed only within the zone of root development, and in our recently reported study of the movement of water in the absence of plants (3) we found no appreciable transfer of water from a moister to a drier portion of a soil when the ratio in the former was as low as 1.5 and that in the latter between 1.5 and 1.0.

If we assume that movement of water through a soil ceases when the ratio in the moistest portion has fallen as low as 1.5; that the deeper subsoil loses water through upward movement only when it is penetrated by plant roots; and, lastly, that plants are able to develop roots into a soil layer only when this has a moisture content above the computed wilting coefficient (5), the ratio 1.5, we must conclude that the roots responsible for the dry condition (indicated by ratios of 1.1–1.4) encountered in any subsoil level either will be found surviving or that they have died only since this level of the subsoil was last reduced to the dry condition.

In order to explain how the dry condition of the deeper subsoil is first established and how it is renewed after wet periods, it seems necessary to assume that among the shallow rooted grasses there are distributed a considerable number of very deep rooted perennials. After this dry condition of the deeper subsoil has once been established it may be maintained through a dry period of several successive years without the presence of any roots in it, the moisture from the rains and snow being held near the surface until it either evaporates or is transpired by the shallow rooted plants, while the upward movement of water from the moist zone beyond the extreme reach of plant roots is at least too slight to show a distinct effect. The absence of the dry condition in the deeper subsoil after prolonged droughts, such as illustrated by field E at Wauneta (table

VI), may be attributed to a temporary absence of the deep rooted perennials or to their fewness. The factors just mentioned are sufficient to account for the maintenance of a dry upper subsoil through which no roots could develop into the moist zone.

The question of whether the living roots are to be found in the deeper subsoil only during each successive wet period, they following the downward extension of the moist zone, continuing to withdraw water until the ratio approximates 1.0, and then dying off, or whether they continue alive but withdrawing practically no moisture throughout the dry periods of several years which intervene between the successive wet periods, is to be answered only by detailed field investigations, involving the use of pits or trenches 12–20 ft. deep.

The present moisture conditions of the deeper subsoil of the prairies, like their plant population, are to be regarded as the result of a slowly established equilibrium, and any alteration of the plant cover may greatly affect the subsoil moisture conditions. The complete suppression of plant life over an acre or more, a condition approached in young orchards and groves kept in clean cultivation, might during a series of wet years raise the moisture content of the deeper subsoil to its water-retaining capacity and maintain this with little change during the ensuing dry period. If such a field were neglected, however, it would soon be taken possession of by many species, most of them shallow rooted annuals, but some deeper rooting perennials, which, meeting little competition for moisture in the deeper subsoil, could develop an extensive root system there and gradually reduce the moisture to approximately the hygroscopic coefficient. Then, as on the prairie, this dry condition would be maintained except at such times as unusually wet seasons extended the moist zone far below its normal limits.

While it is evident from table VI that the lower limit of the dry zone in the deep loessial soils in the semi-arid region is more than 12–15 ft. below the surface we have no data showing its maximum depth. ROTMISTROV (9), from his studies near Odessa, concluded that there permanently moist subsoil in waste land occupied by weeds, etc., is first encountered at 14–30 ft. The depth to which the root systems of the deeper rooted prairie plants indicated by

SHANTZ (10) extend would not suffice to explain the dry condition of the deeper subsoil which we encountered.

The persistently moist condition of the deeper subsoil of the humid prairies is to be attributed to the fewness of the roots developed in them. When deep rooted perennial plants such as alfalfa or forest trees are introduced, their subsoil moisture is utilized to a much greater depth. It is evident that on these a forest once established should be able to maintain itself if protected from fires. The subsoil moisture conditions in general would indicate that the natural condition of grassland in eastern Nebraska is due to other causes than soil moisture conditions, while in western Nebraska it may be fully accounted for by those alone.

The distribution of carbonates in the first 6 ft. of soil in the prairies at McCook and Wauneta indicates that in prehistoric times the climate was similar to that now prevailing (4). Carbonates are found in the surface foot or two only in almost negligible quantities, while in the fourth, fifth, and sixth feet they constitute from 3 to 6 per cent of the weight of the soil.

Summary

1. During a 6-year period, in which the weather was exceptionally favorable for a study of the minimum moisture content of the subsoil, moisture studies were carried out on Nebraska prairies, both in the buffalo-grass formation in the southwestern part of that state, where the climate is typically semi-arid, and in the prairie-grass formation near Lincoln, which lies within the limits of the humid region. The fields were sampled to a depth of 6 ft. or more, and in the case of every sample the hygroscopic coefficient as well as the moisture content was determined, and the moisture condition is expressed as the ratio of moisture content to hygroscopic coefficient, this having the advantage of expressing the *relative moistness* while at the same time indicating whether either free water (1.1 or above) or growth water (1.6 or above) is present, and if so the amount of each.

2. The subsoils of the semi-arid prairies were characterized by their persistent dryness. Usually throughout more or less of the

first 6 ft. a ratio of 1.5 or lower was found, and commonly in one or more of the foot sections a ratio as low as 1.1 was encountered. After droughts of unusual severity the whole of the subsoil to a depth of 6 ft., and in some cases of 12 ft., showed a ratio of approximately 1.0.

3. There was no appreciable further reduction of the moisture content when, after the subsoil had been reduced to this very dry condition, there followed a 4 or 5-month period of practically rainless autumn and winter weather. After such droughts the surface foot was found but little drier than the subsoil.

4. The subsoils of the humid prairies, on the contrary, showed no distinct reduction of the moisture content through a greater depth than 5 ft., and even in this a ratio as low as 1.2 or 1.3 appeared only under the severest drought conditions. The normal moisture condition in the deeper subsoil (6–20 ft.) appears to correspond to a ratio lying between 2.0 and 2.4.

5. The dry condition of the deeper subsoil so common in the semi-arid prairies is to be attributed to the presence of perennials with a vertical root range of 15 ft. or more, while the moist condition characteristic of that of the humid prairies is regarded as evidence that the roots of the native vegetation are but little developed below the fifth foot. The occurrence of areas in the semi-arid prairies, even after a severe drought, in which the subsoil below the sixth foot is quite moist, is to be attributed to the absence or fewness of deep rooted perennials in such places.

6. After the subsoil at any level has been exhausted of the water in excess of the hygroscopic coefficient it remains in this dry condition until the precipitation conditions are sufficiently favorable to raise the ratio to 2.0 or upward throughout the whole distance from the surface down to the level in question. Accordingly during many wet periods following droughts the upper moistened portion of the subsoil will be isolated from any deeper lying moist layer by a zone in which the subsoil is too dry to permit of the penetration of plant roots.

7. While in the semi-arid prairies after protracted droughts the moisture conditions in the first 6 ft. are quite uniform, under more

normal weather conditions they vary much from place to place, thus rendering the results obtained in single borings unreliable as an index of the general moisture conditions.

UNIVERSITY FARM
ST. PAUL, MINN.

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NOTES ON NORTH AMERICAN TREES. IV

C. S. Sargent

Picea glauca (Moench) Voss, Mitt. Deutsch. Dendr. Ges. **16**:93. 1907.—Although *Abies canadensis* Miller is the oldest name for the white spruce, *Picea glauca*, according to the rules of the Vienna Congress, must be adopted for this tree, for there is a *Picea canadensis*, which is a valid name for the hemlock spruce under the genus *Picea*. The Rocky Mountain variety then becomes

Picea glauca var. **albertiana**, n. comb.—*Picea canadensis* var. *albertiana* Rehder, Mitt. Deutsch. Dendr. Ges. **24**:213. 1916.

Juniperus utahensis var. **megalocarpa**, n. var.—*Juniperus megalocarpa* Sudworth, Forestry and Irrigation **13**:307. *figs. 1* and *2*. 1907; Wooton and Standley, Contrib. U.S. Nat. Herb. **19**:37. 1915.—*Sabina megalocarpa* Cockerell, Muhlenbergia **3**:143. 1907. —Differing from the type in its larger fruit sometimes 1.7 cm. in diameter, and in its habit of sometimes forming a single erect stem.

A tree 10–14 m. high, with a straight trunk occasionally 1–1.2 m. in diameter.

Valley of the San Francisco River near Alma, southwestern New Mexico, *W. R. Mattoon*, September 1906, *Alfred Rehder*, August 13 and 14, 1914 (nos. 285, 285b, 289); Arizona: rim of the Grand Canyon, *C. S. Sargent*, September 9, 1904; Angel, near Flagstaff, *Percival Lowell*, September 4, 1910.

Mr. Rehder visited the type station of this tree in New Mexico in August 1914 and obtained a large amount of material which shows that it must be considered a variety of *J. utahensis*, for he found trees with fruit intermediate in size between that of the typical *J. utahensis* (6–7 mm. in diameter) and the largest fruits of *J. megalocarpa*, that the trees with single stems did not always produce large fruit, and that the large-fruited trees sometimes had the characteristic habit of *J. utahensis*. Like that of *J. utahensis*, the fruit of the variety ripens at the end of the second season. A specimen of *J. utahensis* in the herbarium of the Arboretum collected by *E. Bethel* at Radium, Colorado, in November 1908 at an elevation of 2300 m., has fruit 1.3 cm. in diameter and should perhaps be referred to the variety.

Populus tremuloides var. **vancouveriana**, n. var.—*Populus vancouveriana* Trelease apud Tidestrom in Piper and Beattie, Fl.

Northwest Coast, 118. 1915.—Differing from the type in its thicker, more coarsely serrate leaves, densely hoary tomentose below when they unfold, villose aments, and in its pubescent or puberulous branchlets and tomentose winter-buds. Leaves broadly ovate to semiorbicular, rounded or slightly cordate at broad base, abruptly short-pointed or rounded at apex, coarsely crenately serrate and sometimes obscurely crispate on the margins, when they unfold covered below and on the petioles with a thick coat of long matted pale hairs and slightly villose above, soon glabrous, and at maturity thick, dark green, lustrous, and scabrate on the upper surface, paler on the lower surface, 8–11 cm. long and broad, with prominent midribs and primary veins; petioles slender, compressed, becoming glabrous, 5–8 cm. in length. Rachis of the staminate inflorescence slightly villose, the pedicels pubescent; disk of the flower puberulous toward the base, flowers otherwise as in the species. Pistillate inflorescence 5–6 mm. long, the rachis, pedicels, and slightly lobed disk of the flower densely villose, becoming pubescent or glabrous on the fruit; ovary conic, pubescent, with a short style, and stigmas divided into narrow divergent lobes. Fruiting aments 8–9 cm. long, the fruit oblong-conical, pubescent or glabrous, 5 mm. long; pedicels not more than 1 mm. in length.

A tree 10–12 m. tall, with a trunk 30–40 cm. in diameter, stout spreading branches forming a round topped head, stout, reddish brown pubescent or puberulous branchlets often becoming glabrous during their first summer, and acute tomentose pubescent or glabrous winter-buds.

Borders of salt marshes on the coast of Vancouver Island, British Columbia, near Sidney, *J. Macoun*, May 3, 1913 (staminate flowers), May 13, 1913 (pistillate flowers), June 13, 1913, April 1914 (pistillate flowers), April 13, 1914 (fruit), May 6, 1914, *C. S. Sargent*, July 27, 1913; by shingle mill near Sidney, *J. Macoun*, April 2, 1913; old brickyard, Sidney, June 22, 1914; near Victoria, British Columbia, *Engelmann* and *Sargent*, August 19, 1880, *J. Macoun*, August 21, 1893 (no. 2131), May 27 and August 31, 1893 (no. 2232), May 28, 1908 (nos. 85714, 8805a), June 4, 1908 (no. 88059), May 5, 1915; Dead Man's Creek, near Victoria, *J. Macoun*, July 23, 1908; Esquimo, Vancouver Island, *J. Macoun*, June 23, 1887; Cape Laza, near Comox, Vancouver Island, *J. Macoun*, July 7, 1915.

Extreme forms of this tree certainly appear distinct from *P. tremuloides* Michaux and its western variety *aurea* Daniels, but the shape of the leaves is not constant; the branchlets and the young leaves are sometimes glabrous or nearly glabrous; on some branches the winter-buds are not tomentose but are

pubescent or glabrous; and the only constant character I can find in the Vancouver Island trees is the presence of the dense covering of hairs on the rachis of the male and female inflorescence and on the disk of the pistillate flower.

Populus arizonica, n. nom.—*Populus mexicana* Sargent, Silva N. Am. 14:73. *pl. 733* (not Wesmael). 1902; Man. 162. *fig. 136.* 1905.—A photograph of the type specimen of *P. mexicana* Wesmael collected by *Berlandier* between Tampico and Rial del Monte in May 1827, kindly sent to the Arboretum by the late CASIMIR DECANDOLLE, shows clearly that the tree with small fruit which is common in the neighborhood of Tucson, Arizona, was wrongly referred to WESMAEL'S species. For the Arizona tree I now suggest the name of *P. arizonica.* It is well distinguished from the other cottonwoods of the United States by the small fruit, which does not exceed 5 mm. in length.

ARIZONA.—Common up to 2200 m. above sea level. Santa Catalina Mountains, Pima County, *C. G. Pringle*, 1881, *A. Rehder*, August 8, 1914 (no. 256); Bear Creek, foot of Santa Catalina Mountains, *A. Rehder*, August 30, 1916 (no. 452); Sabino Canyon, Santa Catalina Mountains, *J. W. Toumey*, February 20, 1894, *A. Rehder*, August 7, 1914 (no. 233), September 1, 1916 (no. 500); near Tucson, Pima County, *Engelmann* and *Sargent*, September 30, 1880, *J. W. Toumey*, February 27, 1894, February 1898, *C. S. Sargent*, February 27, 1894, March 27, 1916; Douglas, Cochise County, *A. Rehder*, August 27, 1916 (no. 447a, a planted street tree); Yavapai County, near Clarkdale, *A. Rehder*, September 11, 1916 (no. 555), near Camp Verde, September 9, 1916 (no. 542), Beaver Creek, near Camp Verde, September 8, 1916 (no. 540), banks of Gannett Creek, Prescott, September 4, 1916; on Salt River, near the Roosevelt Dam, Gila County, *W. H. Goddard*, June 1917; Coconino County, Sycamore Canyon, *Percival Lowell*, September 1915, *A. Rehder*, September 14, 1916 (no. 573); Hermit Creek, Grand Canyon of the Colorado, *Alice Eastwood*, April 10, 1917 (no. 6002); Turkey Creek, near Flagstaff, *C. O. Lampland*, March 1917; Canyon Diablo, *C. O. Lampland*, April 5, 1915 (these specimens have only very young flower buds and may belong to another species).

CALIFORNIA.—A sterile branch collected by *A. Rehder* July 23, 1914 (no. 128), on Mill Creek above Forest Home, San Bernardino Mountains, is doubtfully referred to this species.

NEW MEXICO.—Near Silver City, Grant County, *E. L. Greene*, 1880 (distributed as *P. Fremontii*), *O. F. Arthur*, March 16, 1918, *M. W. Talbot*, April 1, 1918, *J. A. Scott*, May 18, 1918.

In the neighborhood of Tucson, where this poplar has been planted in considerable numbers, it is a magnificent tree 20–28 m. in height with a trunk

sometimes 1 m. in diameter, a broad head of wide spreading branches, slender branchlets, glabrous or puberulous, and lustrous yellow-green leaves often puberulous early in the season. The bark of the branches and young stems is nearly white and on old trunks it is pale green and slightly divided into broad flat ridges.

Populus arizonica var. **Jonesii,** n. var.—Differing from the type in the pubescent, not puberulous, young leaves, petioles, and young branchlets.

Mexico, Valley of Palms, *Marcus E. Jones*, April 8, 1882 (no. 373, type); valley near Chihuahua, *C. G. Pringle*, March 31, 1886 (no. 885); Saltillo, *C. G. Pringle*, June 4, 1888 (no. 2098, with larger leaves and more pubescent branchlets), *C. S. Sargent*, March 1887 (a very large tree with pendulous branches); Valley of Mexico, *C. G. Pringle*, February 13, 1899 (no. 8019); Pedras Negras, *C. S. Sargent*, March 21, 1900 (a planted tree).

Populus Palmeri, n. sp.—Leaves thin, ovate, cuneate or rounded at the broad base, gradually or abruptly contracted at apex into a narrow acuminate entire point, finely serrate with incurved teeth, ciliate on the margins when they unfold, otherwise glabrous, 6–10 cm. long and 4.5–8 cm. wide; petioles slender, glabrous, 3.5–6 cm. in length. Flowers not seen. Fruit in slender glabrous aments 12–15 cm. long, ovate, obtuse, slightly pitted, puberulous, thin-walled, 4-valved, 6–7 mm. long, the disk deeply lobed, 4–5 mm. in diameter; pedicels slender, 7–9 mm. in length.

A tree 20–21 m. tall with a straight trunk 1 m. in diameter, erect, smooth, pale branches forming an open pyramidal head, the lower branches smaller, horizontal or pendulous, and slender, glabrous branchlets light reddish brown early in the season, becoming pale grayish brown in their second year. Bark pale, 5 cm. thick, deeply divided by wide fissures into narrow ridges.

In most fertile soil near springs, at the base of high chalk bluffs of Nueces Canyon of the upper Nueces River, Uvalde County, Texas, growing with *Salix nigra* var. *Lindheimerii*, *Carya pecan*, *Morus rubra*, and *Ulmus crassifolia*, *E. J. Palmer*, April 11 and September 1918 (nos. 13340, 14511).

In the shape of the leaves and their serration, in the small fruit, and in the remarkably slender branchlets this poplar is so different from all other American species that, although it is still very imperfectly known, I venture to describe it. It is the only species seen by Palmer in Uvalde County.

Populus texana, n. sp.—Leaves thin, glabrous, broadly ovate, truncate at base, gradually narrowed, long-pointed, acuminate at apex, coarsely crenately serrate below the middle, entire above,

7–8 cm. long and 6–7 cm. wide; petioles slender, compressed, 4–7 cm. in length. Flowers not seen. Fruit in slender, glabrous aments 7–8 cm. long, oblong-ovate, acute, deeply pitted, glabrous, thin-walled, 3-valved, 8–9 mm. long, the disk slightly lobed, 2.5–3 mm. in diameter; pedicels slender, 3–4 cm. in length. Seeds ovate, acuminate, 4 mm. long.

A tree up to 20 m. high, with a trunk sometimes 1 m. in diameter, and stout more or less pendulous branches, stout, glabrous, pale yellow-brown branchlets, and acuminate, glabrous winter-buds.

In canyons and along the streams of northwestern Texas, where it appears to be the only cottonwood. Low sandy banks of the Canadian River, Canadian, Hemphill County, *E. J. Palmer*, June 17, 1918 (no. 14107); creek banks, Amarillo, Potter County, *E. J. Palmer*, July 13, 1917 (no. 12541); canyon, Paloduro Creek, Randall County, *E. J. Palmer*, October 3, 1918 (no. 14591); river banks in canyon, Gamble's Ranch, Armstrong County, June 6, 1918 (no. 13959). "One of the largest trees found in Paloduro Canyon, growing in the protection of high bluffs. It usually grows in the protection of high bluffs or at the heads of canyons. The young trees here are slender and straight, but older specimens are very irregular or unsymmetrical in growth, with pale or dark ashy bark. It is rarely found in the more open parts of the canyon here, but near Canyon City it grows on the river margins" (E. J. P. in litt.); Post, Garza County, *E. J. Palmer*, May 31, October 1, 1918 (nos. 13848, 13853, 14575); along creeks, Sweet Water, Nolan County, *E. J. Palmer*, October 21, 1917, May 28, September 28, 1918 (nos. 13045, 13799 type, 13899, 14526).

By the shape of the leaves and by the thickness and color of the branchlets this species cannot be distinguished from *P. Wislizenii* Sargent, but from that species it is well distinguished by the smaller fruit on much shorter pedicels and by the glabrous winter-buds. The range of the two trees is also quite different.

Populus Macdougallii Rose, Smithsonian Misc. Coll. 61:61. 1913.—This species, of which I have not seen the flowers, is well distinguished from *P. Fremontii* by the minute disk of the fruit, which does not exceed 3 mm. in diameter. The fruit is borne on slender, glabrous pedicels 3–5 mm. in length, in racemes 5–6 cm. long; it is ovate and acute at apex to ellipsoidal and acute or acuminate at ends, glabrous, slightly pitted, thin-walled, 3-valved, 10–12 mm. in length. The seed is oblong-ovate, acuminate, 3 mm. in length.

It is probably always a small tree with erect branches and slender branchlets pubescent or puberulous when they first appear, soon becoming glabrous and pale yellow-brown at the end of their first season.

This is the common and probably the only cottonwood of the valley of the lower Colorado River. It is common on both banks of the river at Yuma, and is planted in some of the towns of the Colorado Desert region like Yuma, Mecca, and Indio. It has also been planted at the Needles on the Colorado River in San Bernardino County, California.

POPULUS FREMONTII S. Watson.—This is the common and only cottonwood of the valleys of northern and central California west of the Sierra Nevada. The leaves are slightly cordate at the broad base and coarsely serrate often with few teeth. The fruit is ovate with a disk about 5 mm. in diameter, on pedicels 3–5 mm. in length.

In San Bernardino County, California, Nevada, Utah, and Arizona poplar trees occur which, although the disk of the fruit is smaller or larger than that of the typical *P. Fremontii*, until better known are best considered perhaps varieties of that species. Three of these forms may be distinguished as follows:

POPULUS FREMONTII var. **Thornberii,** n. var.—Leaves broadly ovate, abruptly contracted into acuminate points, slightly cordate at the wide base, coarsely crenately serrate with numerous teeth, glabrous, 6–8 cm. long and broad; petioles 3.5–4 cm. in length. Flowers not seen. Fruiting aments 5–6 cm. long, the capsules ellipsoidal, 3-valved, deeply pitted, 8–9 mm. long; disk 3 mm. in diameter; pedicels 2–3 mm. in length.

A large tree with pale deeply furrowed bark and pale gray glabrous branchlets.

Low ground near Tucson, Pima County, Arizona, *C. S. Sargent*, March 27, 1916.

From the typical *P. Fremontii* this variety differs in the more numerous serratures of the leaves, in the ellipsoidal, not ovate, fruit with a smaller disk, and in the much shorter pedicels. This tree was shown to me by Professor J. J. THORNBER of the University of Arizona, whose name I venture to associate with it.

POPULUS FREMONTII var. **pubescens,** n. var.—Differing from the type in its more pubescent branchlets.

This is a common tree in San Bernardino and San Diego counties, California, and extends into Nevada and southern Utah. The branchlets of the type specimen of *P. Fremontii*, which was collected by *Fremont* in the upper Sacramento Valley, are described as slightly pubescent, but on the other specimens of this tree which I have seen from California north of San Bernardino County they are glabrous, and as the range of the trees with the

distinctly pubescent branchlets extends far beyond the region occupied by typical *P. Fremontii* it will perhaps be best to consider that they represent a geographical variety. I have seen the following specimens:

CALIFORNIA.—San Bernardino Mountains, *G. R. Vasey*, 1880; San Bernardino, *C. C. Parry*, April 1883, *C. S. Sargent*, March 30, 1916; Barstow, San Bernardino County, *W. L. Jepson*, May 1914 (no. 5894), March 8 and 22, 1916 (nos. 6610, 6611, 6626); Warner Hot Springs, San Diego County, *Alice Eastwood*, April 9, 1913 (no. 2619); Bernardo, San Diego County, *Le Roy Abrams*, May 2; 1903 (no. 33701).

NEVADA.—"Kiernan, Meadow Valley, Wash.," *L. N. Goodding*, April 28, 1902 (no. 634); south base of Mount Grant, Mineral County, *A. A. Heller*, July 2, 1913 (no. 10909).

UTAH.—St. George, Washington County, *M. E. Jones*, March 30, 1880 (no. 1611).

POPULUS FREMONTII var. **Toumeyi,** n. var.—Differing from the type in the cordate-cuneate broad base of the leaves, and in the larger disk of the fruit. Leaves ovate, the base shallow cordate and gradually narrowed and cuneate to the insertion of the petiole, gradually narrowed and acuminate at the entire apex, coarsely and irregularly crenately serrate below, glabrous, 6–7 cm. long and broad. Fruit oblong-ovoid to slightly obovoid, acute or obtuse at apex, 8–10 mm. long, the disk 6–7 mm. in diameter; pedicels 4–5 mm. in length.

ARIZONA.—Tucson, Pima County, *J. W. Toumey*, April 28, 1894 (type); Pima Canyon, Santa Catalina Mountains, Pima County, *J. J. Thornber*, March 2, 1913; Santa Cruz River bottoms, Pima County, *J. J. Thornber*, March 30, 1913; Nogales, Santa Cruz County, *McPherson*, April 15, 1915; Hermit Creek, Grand Canyon of the Colorado, Coconino County, *Alice Eastwood*, April 10, 1917 (no. 6002).

Populus Parryi, n. hyb. (*P. Fremontii*×*trichocarpa*).—Leaves ovate, rounded or slightly cordate at the broad entire base, gradually narrowed, acuminate and entire at apex, finely crenately serrate below, sparingly villose and ciliate on the margins when they unfold, soon glabrous, and at maturity thin, dark green, and lustrous above, silvery white below, 6–8 cm. long and broad; petioles slender, slightly compressed, glabrous, 4–6 cm. in length; leaves on vigorous shoots sometimes oblong-ovate, truncate or rounded at base, acute at apex, more coarsely serrate, 9–12 cm. long and 8–10 cm. wide, with stout compressed petioles 3–4 cm. in length.

Staminate aments densely flowered, 5–6 cm. long, puberulous, the bract of the flower broadly obovate, laciniate; anthers 10 or 12; aments of pistillate flowers villose, 6–7 cm. long, becoming at maturity 15–16 cm. in length. Disk of the flower broad, entire or erose on the margin; ovary broad, ovate, puberulous; stigma 3-lobed or occasionally 2-lobed. Fruit broadly ovate, rounded at apex, slightly pitted, puberulous, thin-walled, inclosed sometimes for one-third of its length in the enlarged disk, 5–6 mm. long, often abortive; pedicels puberulous, 2–2.5 mm. in length; seeds narrow-obovoid to ellipsoidal, 3 mm. long.

A large tree with deeply furrowed bark, wide spreading branches, slender glabrous branchlets reddish brown in their first season, light orange-brown in their second year, and acuminate, lustrous, glabrous winter-buds.

Streets of San Bernardino, San Bernardino County, California, *C. C. Parry*, March and April 1883 (type), *C. S. Sargent*, March 16, 1916, *S. B. Parish*, October 15, 1917; along Cottonwood Creek, west side of Owen's Lake, Inyo County, California, *F. V. Coville* and *F. Funston*, June 19, 1881 (no. 996); in the Canoda de las Uvas, about 2 miles north of Fort Tjon, Kern County, California, *F. V. Coville* and *F. Funston*, July 5, 1891 (no. 1163).

These trees appear intermediate in character between *P. Fremontii* and *P. trichocarpa*. The leaves resemble in shape those of the common Californian form of *P. Fremontii*, but are silvery white below like those of *P. trichocarpa* and the other balsam poplars, and their serration is much finer than that of the leaves of *P. Fremontii*, but coarser than that of the leaves of *P. trichocarpa*. The staminate flowers have fewer stamens than those of either of the supposed parents; the disk of the female flowers is very similar to that of both of them, but the ovary, which is glabrous in *P. Fremontii* and densely tomentose in *P. trichocarpa*, is pubescent. PARRY, who first noticed this tree and who would have considered it a new species if he had seen it growing wild, thought that it might have been an exotic species introduced into San Bernardino. The leaves on the specimens of the wild plants from the western base of the Sierra Nevada are similar to those of the San Bernardino trees, but the fruit is rather longer and more acute. Of these specimens COVILLE wrote me November 4, 1892: "I send you by mail specimens of a poplar collected along streams flowing from the southern Sierra Nevada. Specimens of *P. Fremontii*, *P. tremuloides*, and *P. trichocarpa* were collected by the Expedition (Death Valley), and these specimens show characters between *P. Fremontii* and *P. trichocarpa*."

OSTRYA VIRGINIANA K. Koch.—The variety of this tree, on which the branchlets, petioles, and peduncles are covered with short erect glandular hairs, may be distinguished as

OSTRYA VIRGINIANA var. **glandulosa,** n. comb.—*Ostrya virginiana* var. *glandulosa* Spach, Ann. Sci. Nat. II. **16**:246. 1841.

From Quebec and Ontario to southwestern New England and western New York, and in eastern Michigan this is the prevailing variety. The two forms occur in New Jersey, Pennsylvania, Indiana, northern Illinois, southwestern Missouri, Oklahoma, and on the high Appalachian Mountains.

Betula Eastwoodae, n. sp.—Leaves broad-ovate to elliptic, crenately serrate except at the cuneate base, thick, glabrous, dark green above, pale below, rather conspicuously reticulate venulose especially on the upper side, 2.5–3 cm. long and 1.6–2.3 cm. wide; petioles slender, glabrous, often tinged with red, 5–7 mm. in length. Staminate catkins usually solitary or in pairs, sessile, 2–3 cm. long, 5 mm. in diameter, their scales broadly ovate, acute and apiculate at apex, pubescent, dark red. Pistillate catkins pendulous on peduncles 8–10 mm. long, cylindric, 1.5–2 cm. in length, 6–7 mm. in diameter, their scales longer than broad, the lobes rounded at the narrow apex, ciliate, the lateral slightly spreading, one-third shorter than the terminal lobe.

A tree rarely more than 6–7 m. tall with a trunk not more than 15 cm. in diameter, covered with close chestnut brown lustrous bark about 5 mm. thick and marked by conspicuous horizontal white lenticels, and slender red branchlets more or less thickly covered with circular white glands.

Roadsides, Hunker Creek, Yukon District, *J. Macoun*, August 4, 1902 (no. 54412); swamps in the town of Dawson, valley of the Yukon, British Alaska, forming jungles with *Betula glandulosa* Michaux, occasional plants of *Betula alaskana* Sargent, and different willows, *Alice Eastwood*, May 22 and June 14, 1914 (nos. 88, 271=88 type, also nos. 6, 7, 58, 69, 533=69, 89, 272–89, 102, 282, 381).

The relationship of this tree is with *B. glandulosa* Michx., from which it differs in the shape and venation of the leaves, in the pendulous fruiting catkins, and in its arborescent habit.

Betula commixta, n. hyb.? (*B. alaskana*×*glandulosa?*).—Leaves broadly ovate to elliptic, acute at apex, broad-cuneate or rounded at base, coarsely serrate with blunt or acute teeth, thin, glabrous, smooth, dark green and lustrous above, pale and lustrous below, 3–4.5 cm. long and broad; petioles 1.5 cm. in length. Flowers not seen. Fruiting catkins erect, 2 cm. long, 6–7 mm. in

diameter, their scales puberulous, the terminal lobe acute, one-third longer than the rounded lateral lobes.

A shrub 2–3 m. tall, with dark brown stems and slender gray-brown branchlets thickly covered with resinous glands.

On the tundra with *B. glandulosa* in the neighborhood of Dawson, British Alaska, *Alice Eastwood*, Ten Mile House, June 25, 1914 (no. 367 type); Twenty-four Mile House, June 27, 1914 (no. 400).

The proper disposition of this plant is doubtful, and it should perhaps be considered a species. The glandular branchlets and slender erect fruiting catkins resemble those of *B. glandulosa* Michaux, but the larger, acute, sharply serrate leaves are not of that species, and if it is a hybrid the size and serration of the leaves can only have been derived from *B. alaskana* Sargent.

CELTIS OCCIDENTALIS L.—On what is usually considered the type of this species the leaves are broadly ovate, acute or short-acuminate at apex, obliquely rounded at base, coarsely or finely serrate, smooth on the upper surface, glabrous or sparingly pilose along the midribs and veins below, thin, not conspicuously venulose; petioles glabrous or rarely puberulous. The fruit is borne on glabrous or rarely puberulous pedicels much longer than the petioles and is subglobose, ellipsoidal, or slightly obovoid, and 9–10 mm. in diameter; the stone is only slightly reticulate. The branchlets are glabrous or occasionally pubescent.

C. occidentalis is distributed from New England to Virginia and westward to Iowa, southwestern Missouri, western and central Kansas, and eastern North Dakota. It is less common and usually a smaller tree than its varieties *canina* and *crassifolia*, and much less widely distributed than the latter. All the forms of *C. occidentalis* are well distinguished by the dark purple fruit, which is larger than that of the other American species; it is borne on longer pedicels than that of our other species, with the exception of that of *C. Douglasii*.

CELTIS OCCIDENTALIS var. **canina**, n. var.—*C. canina* Rafinesque, Am. Monthly Mag. 2:43. 1817; Planchon, DeCandolle Prodr. 17:174. 1873; Britton and Shafer, N. Am. Trees, 355. 1908.—*C. occidentalis* Sargent, Silva N. Am. 7:67 (in part, *pl. 317*, not Linnaeus). 1895; Hough, Trees N. States and Canada, 193 (in part, *fig. 217*). 1907.—Differing from the type in the usually narrower long-acuminate leaves.

Extreme forms of this variety look very distinct, but trees with leaves intermediate between these and those of the typical form are common. The

fruit varies as in the type from subglobose to obovoid, and there seems little difference in the length of the pedicels, which are always longer than the petioles. The leaves are usually glabrous, but on some of *Bush's* Missouri specimens the midribs and veins are pilose on the lower surface and the petioles are pubescent, as in the variety *crassifolia* (Monteer, nos. 548, 4725; Christian County, no. 4664; Dumas, no. 5905). This variety is distributed from the Province of Quebec to Iowa, Nebraska, North Dakota, and southwestern Missouri, southwestern Oklahoma, New York, and Ohio, and to northwestern Georgia (Cobb County, *R. M. Harper*, no. 166 in Herb. Gray). More distinct is

CELTIS OCCIDENTALIS var. CRASSIFOLIA Gray, Man. ed. 2, 397. 1856.—*C. crassifolia* Lamarck, Encycl. Méth. 4:138. 1797.—Differing from the type in its usually narrower, acuminate, thicker leaves, often more coarsely serrate or nearly entire, scabrate on the upper surface and pilose below along the midribs and veins.

In this form the petioles are usually villose-pubescent, but occasionally are quite glabrous; the pedicels are slightly villose, and the branchlets are glabrous or pubescent.

I have seen specimens of this variety from Viriginia and West Virginia; North Carolina, *A. Gray*, Painted Rock, French Broad River, 1843 (in Herb. Gray); river banks, Biltmore (ex herb. Biltmore no. 1210, with nearly entire leaves); Nashville, Tennessee; southern Indiana, wooded bluff of Blue River, near Middletown, Crawford County, *C. C. Deam*, June 15, 1915 (no. 16423, with coarsely serrate leaves, "a flat-topped shrub about 8 ft. high"), June 25, 1915 (no. 16418, the leaves entire or furnished with occasional teeth, "a shrub 8 ft. high in the dense shade of walnut and buckeye trees"); wooded bluff of the Ohio River, 6 miles east of Cannelton, Perry County, *C. C. Deam*, June 29, 1915 (no. 16627, a tree 8 m. high with nearly entire leaves); southern and western Illinois; Fort Snelling, Minnesota; northern and southern Missouri; central Kansas; eastern and northwestern Oklahoma; Thomas County, central Nebraska; Bigstone, eastern South Dakota; central North Dakota; from the Tongue River Canyon, Big Horn Mountain, Wyoming; from the Black Canyon of the Boise River and the valley of the Clearwater River, Nez Perce County, Idaho; from Berlin, Dallas County, Alabama, *R. S. Cocks*, 1913 (with nearly entire leaves), and from Larissa, Cherokee County, *B. F. Bush*, April 30, 1909 (no. 5561); and Livingston, Polk County, Texas, *E. J. Palmer*, October 9, 1914 (no. 6785).

CELTIS DOUGLASII Planchon, Ann. Sci. Nat. III. 10:293. 1848; Piper, Contrib. U.S. Nat. Herb. 2:221 (Fl. Washington). 1906; Britton and Shafer, N. Am. Trees, 359. *fig. 319*. 1908.—*C. reticulata* Howells, Fl. N.W. America 602 (not Torrey). 1897.—*C. rugosa*

Rydberg, Bull. Torr. Bot. Club 39:304 (not Newberry). 1912.—*C. rugulosa* Rydberg, Fl. Rocky Mountains, 207. 1061. 1917.—This tree, which has sometimes been considered the same as the more southern *C. reticulata* Torrey, can be distinguished from that species by its rather thinner, oblong-ovate, long-acuminate, coarsely serrate leaves, cordate or obliquely cordate at base, glaucescent on the lower surface and glabrous or sparingly pilose on the under side of the midribs and veins, by the slightly pilose petioles, and by the much longer pedicels of the fruit sometimes up to 1.5 cm. in length. The fruit, which has been described as "black or brownish," is light orange-brown on the Oregon and Colorado trees, and is subglobose to ellipsoidal and 7–8 mm. in diameter.

C. Douglasii is a shrub or a small tree rarely 10 m. high, with rough, red-brown, or in Colorado dark gray bark 2.5 cm. thick and irregularly ridged, glabrous or slightly pilose branchlets, and pubescent and tomentose winter-buds.

Nowhere common, it is widely distributed on dry ridges and the rocky banks of streams, and occurs in Oregon east of the Cascade Range in the valley of the Deschutes River, on the rocky banks of the Columbia near the Dalles and on Pine Creek, Gilliam County, and in western Washington ranges from the valley of the Columbia in Klickitat County to the rocky banks of Snake River in Whitman County, and to Big Willow Creek, Canyon County, Idaho; it inhabits the western foothills of the Wasatch Mountains of Utah, south-eastern Utah (Grand River Canyon below Moab, Grand County), the southern slope of the Grand Canyon, Arizona (*A. Rehder*, July 19, 1914, no. 103), "in sand at the mouth of a dry canyon one-half mile below Democrat Springs, Kern River," Kern County, California, *Mrs. Leo Polkingham*, September 1916 (in Herb. Dudley), and on the eastern foothills of the Rocky Mountains of Colorado.

In the shape of the coarsely serrate leaves and in the long pedicels of the fruit *C. Douglasii* is related to *C. occidentalis* var. *crassifolia*, from which it differs in its thicker leaves with conspicuous reticulate veinlets and usually glaucescent and less pubescent on the lower surface, and in the color of the fruit. In the thick reticulate-venulose leaves rough on the upper surface it resembles *C. reticulata*. Geographically *C. Douglasii* is intermediate between *C. occidentalis*, which in its var. *crassifolia* reaches northern Idaho, and *C. reticulata*, which extends northward to the Grand Canyon in Arizona.

CELTIS LINDHEIMERII K. Koch apud Engelmann, Dendr. 2:434. 1872.—*C. Helleri* Small, Bull. Torr. Bot. Club 24:439. 1897;

Britton and Shafer, N. Am. Trees 358. *fig. 318.* 1908; Mackensen, Trees and Shrubs of San Antonio, 17. *pl. 3.* 1909.—KOCH's description of *C. Lindheimerii* was made from a tree growing in the Botanic Garden in Berlin which had been raised from seeds gathered at New Braunfels, Texas, by LINDHEIMER and sent by ENGELMANN to Berlin. KOCH's description leaves no doubt that *C. Lindheimerii* (Engelmann in Herb. A. Braun) is the tree with leaves pale and densely pubescent on the lower surface and pubescent branchlets which is common at New Braunfels and in the neighborhood of San Antonio.

Of the specimens of this tree which I have seen the oldest was collected by *Drummond* in 1834 without locality but probably near Austin (nos. 343?, 334, 259 in Herb. Gray). It was collected by *Lindheimer* at New Braunfels, Comal County, in 1850 (no. 444 in Herb. Gray). *Mohr* collected it in the valley of the Comal River, New Braunfels, in 1850. I collected it at San Antonio, Bexar County, in 1881, and *Bush* also collected it at San Antonio October 1900 (no. 1246), September 1901 (no. 797), and March 1902 and 1903 (nos. 1172, 3677). *Palmer's* collections of this tree are from Sutherland Springs, Wilson County (no. 9302), Goliad, Goliad County (no. 9128), San Marcos, Hays County (no. 13311), dry limestone banks, South Llano River, Telegraph, Kimble County (no. 10931).

C. Lindheimerii is most abundant in the neighborhood of streams and springs and occurs less commonly on higher ground. I have no evidence that it grows on the Edwards Plateau or westward.

CELTIS RETICULATA Torrey, Ann. Lyc. N.Y. 2:247. 1828; Planchon, Ann. Sci. Nat. III. 10:293. 1848.—*C. occidentalis* var. *reticulata* Sargent, Forest Trees N. Am. 10th Census U.S. 9:126. 1884; Garden and Forest 3:40. *fig. 12.* 1890.—*C. mississippiensis* var. *reticulata* Sargent, Silva N. Am. 7:72 (in part). 1895; Man. 301. *fig. 243.* 1905.—*C. reticulata*, *C. Lindheimerii*, and *C. Douglasii* are similar in their thick leaves, rough on the upper surface and conspicuously reticulate venulose below, and in their pedicels longer than the petioles. The entire leaves green on the lower surface and the orange-red fruit on shorter pedicels of *C. reticulata* distinguish it from *C. Douglasii*. The shape of the leaves of *C. Lindheimerii*, pale and pubescent below over their whole surface, makes it easy to distinguish that species from *C. reticulata*.

I have not seen the flowers and spring leaves of *C. reticulata*, which, although it was described 90 years ago, is still very imperfectly known. The young

leaves, like those of the other species of this group, probably show little evidence of the prominent veinlets which are not conspicuous on them before early summer. In collections of Texas plants *C. Lindheimerii* and more frequently *C. laevigata* var. *texana* have been confounded with *C. reticulata*.

Seedlings raised at the Arboretum from seeds of a tree with rough, mostly entire leaves growing at the Montezuma Well in central Arizona (*Rehder*, no. 537) have coarsely serrate leaves which are rough or nearly smooth above on the same branchlet.

From western Texas I have seen specimens of *C. reticulata* collected in Uvalde, Kimble, Mitchell, Nolan, Callahan, Randall, Hartley, and Jeff Davis counties. It ranges into western Oklahoma, and through southern New Mexico to southern, central, and northeastern Arizona, and occurs on Cedros Island off the coast of Lower California (*Veatch*, 1872, in Herb. Gray).

CELTIS RETICULATA var. **vestita**, n. var.—Differing from the type in its more pubescent serrate leaves and more pubescent petioles. Leaves broadly ovate, acute or acuminate at apex, unsymmetrically rounded or subcordate at base, the margins thickened, ciliate, and sharply but irregularly serrate, thick, dark green and scabrate above, paler and coated below with short pale pubescence with longer hairs on the slender midribs, primary veins, and conspicuoulsy reticulate veinlets, 3.5–4.5 cm. long and 3–3.5 cm. wide; petioles densely tomentose, 4–5 mm. in length; leaves on vigorous shoots acuminate, mostly cordate at base, more coarsely serrate, rugose, and covered above with short white hairs and more densely pubescent below, 6–8 cm. long and 4–4.5 cm. wide, their petioles thickly covered with matted pale hairs, 8–9 mm. in length. Fruit orange-red, 6–7 mm. in diameter.

A small tree with a trunk 20–25 cm. in diameter and slender pubescent branchlets, those of vigorous shoots stouter and densely villose.

Near Canton, Blaine County, Oklahoma, in low ground along the North Fork of the Canadian River, *D. M. Andrews*, August 15, 1915 (nos. 21, 49 type).

Specimens of vigorous sterile branches collected by *Palmer* at Canadian, Hemphill County, Texas, June 17, 1918 (no. 14109), may prove a distinct form of this variety. The leaves are acuminate, obliquely cordate at base, coarsely serrate, more pubescent below, 6–9 cm. long and 3.5–5 cm. wide; the petioles and branchlets are densely tomentose.

CELTIS LAEVIGATA Willdenow, Berl. Baum. ed. 2. 81. 1811.—*C. mississippiensis* Bosc in Encycl. Mét. Agric. 7:577 (*nomen nudum*). 1821; Spach, Ann. Sci. Nat. II. 16:42. 1841.—*C. Berlandierii* Klotzsch, Linnaea 20:541. 1847; Parlatore, DeCandolle

Prodr. 17:178 (in part). 1873.—For this tree of the southern states the name *C. mississippiensis* has usually been adopted. Bosc published a brief account, without a name or technical description, of Le Microcoulier de la Louisiana cultivated in France in the *Nouveau Cours Complet d'Agriculture* (8:529. 1809), and republished it in the second edition of this work (10:41. 1822). As his plant came from near the mouth of the Mississippi River there is little doubt of its identity with the *C. mississippiensis* of Spach, for it was Spach who first described this tree as *C. mississippiensis*, Bosc's earlier *C. mississippiensis* being a *nomen nudum* and 10 years later than *C. laevigata* of Willdenow which, following K. Koch, must be taken up for our tree. A cotype of *C. Berlandierii* Klotzsch (no. 2318), collected by *Berlandier* at "Matamoras de Tamaulipas," April 1831, is preserved in the Gray Herbarium on a sheet with *Berlandier's* no. 885, also collected at Matamoras in April 1831. A fragmentary specimen with flowers, collected by *Berlandier* in February 1828 (no. 1487–2271), a fruiting branch without locality or date and numbered 2429 on a label printed for the Gray Herbarium, and a vigorous shoot (no. 2429–999), collected by *Berlandier*, May 1824, "De Goliad à Bexa," are mounted on another sheet. The leaves and fruit of these *Berlandier* specimens only differ in their smaller size from specimens of the leaves and fruit of *C. laevigata* grown on the rich bottom lands of the Mississippi Valley, a difference which the dryness of the region where *Berlandier* collected them explains.

C. laevigata, when it grows under favorable conditions, is a tree sometimes 30 m. high, with somewhat pendulous branches and slender, glabrous, red-brown branchlets. The leaves are thin, usually oblong-lanceolate, long-pointed and acuminate at apex, unsymmetrically rounded and often oblique or cuneate at base, frequently more or less falcate, entire or furnished with a few teeth usually toward the apex, green on both surfaces, glabrous, smooth or occasionally scabrate above. The fruit is bright orange-red on pedicels shorter or slightly longer than the petioles.

C. laevigata is distributed from the coast of Virginia to the Everglade Keys of southern Florida and through the Gulf states to the valley of the lower Rio Grande in Nuovo Leon, Mexico, and through eastern Texas and Arkansas to

eastern Oklahoma and Kansas, northern Missouri, southern Illinois, and southwestern Indiana; also in Bermuda. Trees occasionally occur with leaves more or less sharply serrate nearly to the base, and this variety may be distinguished as

CELTIS LAEVIGATA var. **Smallii,** n. var.—*C. Smallii* Beadle, Small Fl. S. United States, 365. 1329. 1903.—Differing from the type only in its constantly serrate leaves.

CELTIS LAEVIGATA var. **texana,** n. var.—*C. texana* Scheele, Linnaea **22**:146. 1847.—*C. Berlandierii* Planchon, DeCandolle Prodr. **17**:178 (in part, not Klotzsch). 1873.—Differing from the type in the shorter ovate to lanceolate thicker leaves often pubescent on the midribs and veins below, in the often more prominent veinlets and pubescent petioles, and in its often pubescent branchlets. Leaves ovate to lanceolate, acuminate, unsymmetrically rounded or cordate at base, entire or sparingly and irregularly serrate, often subcoriaceous, dark green, smooth and granulate or rarely scabrate above, green below, with slender midribs and primary veins glabrous or sparingly villose-pubescent and furnished with small axillary tufts of pale hairs, and thin, only slightly raised, reticulate veinlets, 3.5–7 cm. long and 2–3.5 cm. wide; petioles slender, pale pubescent, 5–7 mm. in length. Flowers not seen. Fruit subglobose but rather longer than broad, dark orange-red, 6–7 mm. in length; pedicels glabrous or puberulous, slightly longer than the petioles.

An arborescent shrub or small tree rarely more than 8 m. high, often growing in groups, with pale or grayish rough bark rarely covered with wartlike excrescences, and slender, reddish, glabrous or gray-brown, pubescent branchlets.

I have taken up SCHEELE's name for the common *Celtis* of the Edwards Plateau and western Texas with some hesitation, for I have not seen his type specimen. His description perfectly applies, however, to a specimen collected by *Lindheimer* at New Braunfels in the herbarium of the Arboretum (no. 4 ex herb. Engelmann as *C. texana*). On other specimens collected by *Lindheimer* at New Braunfels the lower side of the midribs and veins of the leaves are sparingly villose-pubescent, and such pubescence is found on the leaves of most of the specimens which I have referred to this variety.

TEXAS.—New Braunfels, Comal County, *F. Lindheimer,* 1850 (nos. 1158, 1159 in Herb. Gray, distributed by the Mo. Bot. Gard. as *C. Berlandierii*); no. 4 (in Herb. Arnold Arboretum ex Herb. Engelmann, type); Comanche

Spring, New Braunfels, *C. Mohr*, December 1880; San Antonio, Bexar County, *B. F. Bush*, October 2, 1900 (no. 1223), September 16, 1901 (no. 807), March 23, 1902 (no. 1174), *B. Mackensen*, October and December 1910; Sutherland Springs, Wilson County, *E. J. Palmer*, March 30, 1916 (no. 9297); Pleasanton, Atascosa County, *E. J. Palmer*, September 23, 1916 (no. 10773); rocky river banks, Blanco, Blanco County, April 6, 1918 (no. 13294), *Berlandier* "entre le rio de las Nueces et Laredo," June 1820 (nos. 601, 2011 in Herb. Gray); Boerne, Kendall County, *E. J. Palmer*, May 18, 1918 (no. 13644, with hoary tomentose branchlets); Kerrville, Kerr County, *B. Mackensen*, May 1, 1910; Uvalde, Uvalde County, *E. J. Palmer*, April 10 and May 6, 1918 (nos. 13324, 13509, with coarsely serrate leaves scabrate on the upper surface); Sweet Water, Nolan County, *E. J. Palmer*, July 6, 1917 (nos. 12432, 12433); Barksdale, Edwards County, *E. J. Palmer*, May 7, 1918 (no. 13519); banks of Devil's River, Valverde County, *E. J. Palmer*, May 14, 1918 (no. 13601); Limpia Canyon, Jeff Davis County, *Tracy* and *Earl*, April 25, 1902 (no. 238 in Herb. Gray); Post, Garza County, *E. J. Palmer*, May 31, October 1, 1918 (nos. 13837, 14565); banks Canadian River, Canadian, Hemphill County, *E. J. Palmer*, June 17, 1918 (no. 14110); Strawn, Palo Pinto County, *E. J. Palmer*, June 27, 1918 (no. 14256); Gamble's Ranch, Armstrong County, June 6, 1918 (no. 13969); along the Brazos, Graham, Young County, *J. Reverchon*, October 27, 1902 (nos. 3262, 3267); dry rocky hillsides, Baird, Callahan County, *E. J. Palmer*, May 26, 1918 (no. 13680); Paloduro Canyon, Potter County, *J. Reverchon*, May 22, 30, 31, 1902 (nos. 3265, 32 f. f. 2930, 2930 bis), *E. J. Palmer*, June 3, 1918 (no. 13866), *C. S. Sargent*, April 1916; Tivoli, Refugio County, *E. J. Palmer*, March 22, 1916 (no. 9250, with broadly ovate coarsely serrate leaves and pubescent branchlets); Austin, Travis County, *G. W. Letterman*, August 23, 1892; Dallas, Dallas County, *J. Reverchon*, August 1860 (no. 854), *B. F. Bush*, September 30 and October 27, 1900 (nos. 1190, 1614, 1615), April 8, 1904 (no. 4254); rocky bluffs, Dallas County, *J. Reverchon*, April 15, 1902; bottom lands Elmo, Kaufman County, *J. Reverchon*, October 22, 1902 (no. 3263); "sandy woods, Forks of the Trinity," Dallas County, *J. Reverchon*, (no. 3268); along the Brazos, Bryan, Brazos County, *E. J. Palmer*, April 26, 1918 (no. 13459); Velasco, Brazoria County, *E. J. Palmer*, March 21, 1918 (no. 13133); Columbia, Brazoria County, *E. J. Palmer*, September 29, 1914 (no. 66760); San Augustine, San Augustine County, *E. J. Palmer*, April 1, 1918 (no. 13245); Houston, Harris County, *E. J. Palmer*, March 19, 1918 (no. 13113); *E. N. Plank*, Eagle, Shelby County.

OKLAHOMA.—Sand hills, Ingersoll, Alfalfa County, *C. S. Sargent*, May 6, 1902; near Page, Leflore County, *G. W. Stevens*, September 1913 (no. 2724); low woods along river, Clinton, Custer County, *E. J. Palmer*, July 16, 1917 (no. 1255).

NEW MEXICO.—*A. Fendler*, without locality, 1847 (no. 775 in Herb. Gray); Berendo Creek, Sierra County, *C. B. Metcalf*, May 23, 1904 (no. 926); bank of creek near Roswell, Chaves County, *A. Rehder*, August 16, 1916 (no. 352).

KANSAS.—Woods, Barber County, *A. S. Hitchcock*, 1896 (no. 815 in Herb. Gray); vicinity of Huntsville, Walker County, *R. A. Dixon*, July 1909 (no. 387 in Herb. Gray).

MISSOURI.—Willard, Greene County, *J. W. Blankinship*, August 2, 1893; Noel, McDonald County, *B. F. Bush*, August 7, October 12, 1908 (nos. 4977, 5255), *E. J. Palmer*, September 5, 1913, May 5, 1914, October 11, 1918, (nos. 4141, 5795, 14665, 14668); "along rocky banks," Carthage, Jasper County, *E. J. Palmer*, 1917, Webb City, *E. J. Palmer*, September 28, 1908; Knight's Station, *C. S. Sargent* and *E. J. Palmer*, October 8, 1911 (no. 3489), July 13, 1913 (no. 4019).

MEXICO.—Reynosa, Tamaulipas, on the lower Rio Grande, *C. G. Pringle*, August 7, 1888 (no. 2082); Saltillo, Coahuila, *Ed. Palmer*, 1898.

CELTIS LAEVIGATA TEXANA f. **microphylla,** n. f.—Differing from the variety in its smaller leaves with more prominent reticulate veinlets, and more densely villose-pubescent petioles. Leaves broadly ovate, acute, unsymmetrically rounded at base, smooth, dark green and granulate above, yellow-green below, with villose pubescent midribs and veins and conspicuous reticulate veinlets, 2–2.5 cm. long and 1.5–2 cm. wide. Flowers and fully grown fruit not seen.

A shrub with slender, red-brown branchlets densely pubescent in their first season, becoming puberulous in their second year.

Rocky banks of streams, Sweet Water, Nolan County, Texas, *E. J. Palmer*, May 27, 1918 (no. 13751 type).

CELTIS LAEVIGATA var. **brachyphylla,** n. var.—Differing from the type in the shorter thicker leaves. Leaves ovate, acuminate and long-pointed at apex, very oblique and rounded or cordate at base, entire, occasionally furnished on one side near the base with a broad rounded lobe, glabrous, thick and firm, green on the two surfaces, 3.5–4 cm. long and 2–3 cm. wide, with slender midribs and veins; petioles slender, glabrous, 5–6 mm. in length. Fruit subglobose to short-oblong, bright orange-red, 6–7 mm. in diameter; pedicels glabrous, longer than the petioles.

A tree about 10 m. tall, with slender, glabrous, dark red-brown branchlets.

Rocky banks of the canyon of the Nueces River near Uvalde, Uvalde County, Texas, *E. J. Palmer*, September 26, 1918 (no. 14517 type).

CELTIS LAEVIGATA var. **anomala,** n. var.—Differing from the type in its oblong-ovate, acute leaves, cordate or unsymmetrically

cordate at base, and dark purplish fruit covered with a glaucous bloom. Leaves entire, glabrous, dark green and scabrate above, paler and conspicuously reticulate venulose below, 2–4 cm. long and 1.5–2 cm. wide; petioles sparingly villose pubescent, 4–6 mm. in length. Fruit short-oblong, 6–7 mm. in length, the pedicels as long as or slightly longer than the petioles.

A shrub from 1.5–2 cm. tall with slender pubescent branchlets.

"In deep sands, among shin oak and *Quercus marylandica*," Clyde, Callahan County, Texas, *E. J. Palmer*, September 30, 1918 (no. 14550, type).

CELTIS LAEVIGATA var. **brevipes**, n. var.—*C. brevipes* S. Watson, Proc. Am. Acad. 14:297. 1879.—Differing from the type in its yellow fruit on shorter puberulous pedicels and in its puberulous petioles. Leaves ovate, acuminate, unsymmetrically rounded or cuneate at base, entire or rarely furnished with occasional teeth, glabrous, dark green and smooth or slightly roughened on the upper surface, yellow-green below with small clusters of pale hairs in the axils of the slender veins, inconspicuously reticulate venulose, 3.5–5 cm. long and 1–2.5 cm. wide; petioles slender, puberulous, 5–7 mm. in length. Flowers not seen. Fruit short-oblong, canary yellow, 5–6 mm. long; pedicels puberulous, shorter or slightly longer than the petioles.

A small tree with slender, glabrous, red-brown branchlets.

ARIZONA.—Camp Grant, Pinal County, *T. J. Rothrock*, July 1874 (no. 337, type of *C. brevipes* in Herb. Gray), *J. G. Lemmon*, 1880 (no. 68 in Herb. Gray)—; along irrigating ditches, Tucson, Pima County, *Engelmann* and *Sargent*, September 24, 1880; banks of Rillito Creek near Tucson, *A. Rehder*, August 7, 1917 (no. 240 type); Apache Trail, Fish Creek, *Alice Eastwood*, April 19, 1917 (no. 6250); Crow Creek Canyon, Chiricahua Mountains, Cochise County, *J. W. Toumey*, July 1894 ("tree 18°×20′–30′"); Tonto Basin, Gila County, *J. W. Toumey*, June 19, 1892.

UTAH.—River Canyon, southeast Utah, *Alice Eastwood*, June 1892.

CALIFORNIA.—Laguna, San Diego County, *D. Cleveland*, July 10, 1885.

The specimens of *C. brevipes* in Herb. Gray and the specimen from San Diego County, California, have only partly grown fruit, but in the shape of the leaves they so closely resemble the yellow-fruited Arizona trees that there can be little doubt that they are of the same variety, although on the Californian specimen the leaves are distinctly rough on the upper surface, as they are in the specimen collected by *Engelmann* and *Sargent* at Tucson.

CELTIS PUMILA Pursh, Fl. Am. Sept. 1:200. 1814; E. J. Hill, Bull. Torr. Bot. Club 27:497. *pl. 33.* 1900.—*C. occidentalis* B *pumila* Muhlenberg, Cat. 100 (*nomen nudum*). 1813; Gray, Man. ed. 2. 397. 1856.—*C. mississippiensis* var. *pumila* Mackensen and Bush, Man. Fl. Jackson County, Missouri, 72. 1902.—Often considered a variety of *C. occidentalis*, *C. pumila* can be separated from that species by its smaller, usually entire, rather thicker leaves, by its small, dark reddish purple fruits on pedicels shorter or only slightly longer than the petioles, by its more deeply pitted nutlet, and shrubby habit. The color of the fruit and its short pedicels indicate a nearer relationship with *C. laevigata* than with *C. occidentalis*.

C. pumila has been found in Pennsylvania, Delaware, and the District of Columbia, western New York, northern Indiana and Illinois, middle Tennessee, northeastern Mississippi, near Augusta, Georgia, near Lake Okeechobee, Florida (*Ed. Palmer*, 1874, no. 515 in Herb. Gray), Missouri, and northeastern Arkansas (Eureka Springs, Carroll County, *E. J. Palmer*, no. 4409).

A branch without fruit, with small, nearly entire, glabrous leaves, collected by *Mohr* on uplands west of Franklin, Alabama, October 8 (no year, no. 66), and described as "a low spreading tree of slender growth," appears in spite of its habit to be *C. pumila*. A glabrous specimen with pedicels as long as or longer than the petioles collected on the sandy seashore at Hillsboro, Florida, by *F. A. Marten* (no. 6506 in Herb. Gray) is probably from a depauperate form of *C. laevigata*.

CELTIS PUMILA var. **georgiana**, n. var.—*C. occidentalis* Abbot and Smith, Insects of Georgia, *pl. 36* (not L.). 1797.—*C. occidentalis* var. *pumila* Chapman, Fl. 417 (not Muhlenberg). 1865.—*C. georgiana* Small, Bull. Torr. Bot. Club. 24:439. 1897; Britton and Shafer, N. Am. Trees, 357, *fig. 316.* 1908.—Differing from the type in the rugose upper surface of the leaves, more or less densely pilose along the midribs and veins below, in the pilose petioles, and puberulous pedicels.

A shrub or small tree occasionally 10 m. tall, with slender pubescent branchlets sometimes becoming glabrous by the end of their first season. The fruit was described as "tan" color by SMALL, and by BRITTON and SHAFER as "red-purple to yellowish." The fruit when fully grown in early summer is dull yellow in color, but by the middle of October it becomes reddish purple like that of *C. pumila*, and is often covered with a glaucous bloom.

C. pumila var. *georgiana* occurs on rocky bluffs, Franklin Furnace, Sussex County, New Jersey (*K. K. MacKenzie*, August 22, 1909, no. 4331), and ranges from the Piedmont region of North Carolina (Raleigh, Wake County, *T. G. Harbison*, June 11, 1918) to western Florida, Autauga and Dallas counties, Alabama, and occurs in southern Missouri (rocky hills and bluffs, *B. F. Bush*, Swan, Taney County, September 23, 1905 [no. 5040], Monteer, Shannon County, August 18, 1901 [no. 703], Noel, McDonald County, August 9, 1908 [no. 5040]). The oldest specimen of this plant which I have seen was collected near Augusta, Georgia, by *Olney* and *Metcalf* in 1855 (in Herb. Gray).

On the rocky wooded slopes and ridges of Lawrence, Orange, Washington, Crawford, Perry, Floyd, and Harrison counties in the extreme southern part of Indiana a dwarf *Celtis* occurs in a few isolated stations. In the general outline of the leaf it resembles *C. pumila*, but the pedicels of the lighter-colored fruits are much longer; the leaves, which are smooth or nearly so on the upper surface and rather thicker than those of *C. pumila*, are slightly pubescent along the under side of the midribs and veins and on the petioles; the branchlets are usually puberulous. Judged by the present inadequate information now accessible concerning this plant it appears intermediate between *C. pumila* and its variety *georgiana*, although the nutlets are smoother than those of *C. pumila*, and it may be distinguished as

CELTIS PUMILA var. **Deamii**, n. var.—Leaves broadly ovate to oblong-ovate, acuminate and often long-pointed at apex, unsymmetrically rounded at base, entire or occasionally sharply and irregularly serrate above the middle, thick, dark green on both surfaces, smooth or slightly roughened above, 3-nerved, reticulate venulose, slightly villose pubescent on the prominent midribs and veins, 6–8 cm. long and 3.5–4 cm. wide; petioles slender, villose pubescent, 8–10 mm. in length; leaves on vigorous shoots acute or acuminate, obliquely rounded at base, thicker, entire, scabrate above, often 10 cm. in length. Flowers not seen. Fruit subglobose, ellipsoidal or slightly obovoid, tan color or orange until after midsummer, becoming when fully ripe dark orange-red, 7–8 mm. in diameter; pedicels 6–15 mm. long.

An arborescent shrub 2–4 m. high, with a stem 3.5–5 cm. in diameter, covered with dark, rough, deeply fissured bark and slender, reddish brown, slightly pubescent branchlets.

C. C. Deam, 6 miles from Derby, Perry County, July 4, 1912 (no. 11502), near Mitchell, Lawrence County, August 16, 1912, September 2, 1915, August 13, 1918 (nos. 12052, 12055, 18474, 18479, 26218), wooded bluffs of the Ohio River west of Leavenworth, September 11, 1915 (nos. 18586, 18589), on a bluff 3 miles south of New Middletown, Harrison County, September 6, 1915 (no. 18727 type), near Big Springs, Washington County, September 12, 1915 (no. 8987), wooded bluff, Indian Creek, near Corydon, Harrison County, August 15, 1918 (no. 26232), near Elizabeth, Harrison County, September 26, 1918 (no. 26798).

I take much pleasure in associating with this plant the name of CHARLES CLEMON DEAM, State Forester of Indiana, who for many years has industriously studied the trees of Indiana.

PERSEA PUBESCENS (Pursh) Sargent.—The first varietal name *pubescens* of PURSH (1814) was taken up for this tree in *The Silva of North America* (7:7. 1895); but under the rules adopted by the Vienna Congress the first specific name must be used, and this is *palustris* (*Tamala palustris* Rafinesque, Fl. Tellur. 137. 1838), and *Persea pubescens* (Pursh) Sargent should become *P. palustris* (Rafinesque) Sargent.

PLATANUS OCCIDENTALIS L.—The leaves of the northern plane tree are broadly ovate, 3–5-lobed by broad shallow sinuses rounded in the bottom, cordate or truncate at base, becoming glabrous except on the under side of the midrib and principal veins, the lobes broad, acuminate, serrate-toothed with long straight or curved remote acuminate teeth. Individual trees with leaves cuneate at base may be distinguished as

PLATANUS OCCIDENTALIS f. **attenuata,** n. f.—Differing from the type in the long cuneate base of the usually less deeply lobed leaves.

I have seen specimens of this form from Selma, Dallas County, Alabama, *T. G. Harbison*, June 31, 1916 (no. 51 type). Mississippi: *T. G. Harbison*, Pelahatchee, Rankin County, May 26, 1915 (no. 14), Jackson and Bolton, Hinds County, June 28, 29, 1916. Texas: *E. J. Palmer*, banks of Peyton's Creek, Matagorda County, May 6, 1916 (no. 9683), rocky creek banks, Boerne, Kendall County, May 19, 1916, Lacey's Ranch, Kerr County, May 31, 1916 (no. 9982). Oklahoma: *G. W. Stevens*, valley of the Chikaskia River near Tonkawa, Kay County. Missouri: *B. F. Bush*, near Monteer, Shannon County, September 1, 1911 (no. 663). Indiana: *C. C. Deam*, low banks of the Wabash River, near Murray, Wells County, May 26, 1916 (no. 19817).

PLATANUS OCCIDENTALIS var. **glabrata,** n. var.—*P. racemosa* Hemsley, Bot. Biol. Am. Cent. 3:162 (not Nuttall). 1882.—*P. glabrata* Fernald, Proc. Am. Acad. 36:493. 1901.—*P. densicoma* Dode, Bull. Soc. Dendr. France 7:67. 1908.—Differing from the type in the 3-lobed leaves, truncate, broad-cuneate or rarely slightly cordate at base. Leaves usually broader than long, truncate, broad-cuneate or rarely cordate at base, 3-lobed by sinuses acute or rounded in the bottom, the lobes long-acuminate, entire, the lateral often furnished near the base with one or rarely with two small acuminate incurved secondary lobes, occasionally found also on the terminal lobe; when they unfold hoary tomentose below and pubescent above; pubescent when the flowers open toward the end of March, and in early summer pubescent along the under side of the midribs and veins but otherwise glabrous, usually 7–14 cm. long and 8–9 cm. wide, their petioles pubescent, becoming glabrous; peduncles with one or occasionally two heads of flowers and fruit.

Described from specimens collected in the Provinces of Coahuila and Nuovo Leon, Mexico, this *Platanus* has been found not to be uncommon in western Texas, where it has been collected by *S. B. Buckley* near Austin (without date or number), by *A. A. Heller*, Kerrville, Kerr County, April 1894 (no. 1622), and by *E. J. Palmer* on the banks of the Llano River at Llano, Llano County, June 23, 1916 (no. 10279), rocky banks of upper Seco Creek, Bandera County, May 18, 1916 (no. 10241), gravel bank, Nueces River, Uvalde, Uvalde County, September 24, 1918 (no. 14480), Fredericksburg Junction and Boerne, Kendall County, June 5 and May 19, 1916 (nos. 9817, 9826, 10069), rocky banks of the Guadalupe River, Kerrville, Kerr County, May 29, 1916 (no. 9921), rocky banks of upper Seco Creek, June 18, 1916 (no. 10241), Utopia and Sabinal, Uvalde County, April 10, 1917, June 7, 1916 (nos. 10100, 11523), rocky banks, Devil's River, Valverde County, October 18, 1916, March 26, 1917 (nos. 11084, 11371), Palliam, Zavalla County, March 21, 1917 (no. 11332).

The close connection of this variety with typical *P. occidentalis* is shown by the appearance on leading shoots of 5-lobed leaves with serrate lobes (*Palmer*, Sabinal, Uvalde County, no. 10100, and Devil's River, Valverde County, no. 110841). More significant perhaps is the fact that occasionally trees occur growing with *P. occidentalis* north of Texas which cannot be distinguished from the Mexican types of *P. glabrata*. Such specimens are those of *E. J. Palmer*, Choctaw County, Oklahoma, July 13, 1916 (no. 10463), the type of *P. densicoma* collected in the Maquoketa River, Jackson County,

eastern Iowa, preserved in Herb. Mus. Paris (photograph Herb. Arnold Arboretum), Biltmore, North Carolina (Herb. Bilt. no. 1271b), and of *John Robinson*, Brookline, Massachusetts, June 1880.

MAGNOLIA VIRGINIANA L.—*M. glauca* L.—This species was based on the *Tulipifera virginiana* Plukenet, Alm. Bot. 379. *pl. 68*, and the *Magnolia foliis ovato-lanceolatis* Linnaeus, Hort. Cliff. 222; Gronovius, Fl. Virg. 61; and the *Magnolia Lauri folia subtus albicante* Catesby, Nat. Hist. Car. 1:39. *pl. 30*.

There are two distinct forms of this tree, one with glabrous branchlets and pedicels and usually narrow leaves, and one with branchlets and pedicels thickly clothed with long, silvery white hairs and often broader leaves. Specimens preserved in the British Museum show that the former is the type of LINNAEUS' species. *Tulipifera virginiana*, etc., of PLUKENET is represented in the Sloan Herbarium by 2 specimens, one in PLUKENET's own herbarium, the other one of a number of plants collected in Maryland by *Dr. Krieg* and a *Mr. Vernon*. The latter, Dr. RENDLE tells me, agrees with PLUKENET's figure and has a glabrous pedicel. Of the *Hort. Cliff.* specimens Dr. RENDLE writes: "We have a specimen labeled *glauca*. The species' names, however, in *Hort. Cliff.* are rarely in LINNAEUS' handwriting and *glauca* is in the usual hand, but there is also written in what may be LINNAEUS' own hand *Magnolia* Catesby, with a reference to t. 39; this suggests that LINNAEUS regarded the *Hort. Cliff.* plant as identical with CATESBY's. The specimen is in flower and has a glabrous pedicel." CATESBY'S specimen in the British Museum has no flower, but his plate plainly shows that the pedicel is glabrous.

The typical *Magnolia glauca* is a small tree rarely more than 10 m. high, or often a shrub. It is the only form which grows from Massachusetts to southeastern Virginia. Farther south it is rare and I have only seen specimens from Newbern, North Carolina, Darlington, Andrews, Bluffton, Georgetown, and Yemassee, South Carolina, and Meldrin, Georgia. Specimens collected in Florida in the vicinity of Eustis Lake, Lake County, by *G. V. Nash* (no. 575) and in the neighborhood of Orlando, Orange County, by *C. H. Baker* and *T. G. Harbison* have petioles, pedicels, and branchlets puberulous. For the form with the pubescent pedicels and branchlets I suggest the name

MAGNOLIA VIRGINIANA var. **australis,** n. var.—Differing from the type in the silky white pubescence on the pedicels and branchlets.

Leaves remaining on the branches until spring without change of color, elliptic to oval, oblong-obovate or rarely lanceolate, with puberulous, pubescent, or tomentose petioles and varying in width from 2.5 to 9.5 cm., trees with the broadest leaves being confined to western Louisiana and eastern Texas. This southern variety of *M. virginiana* is a tree often 20–30 m. high with a tall trunk occasionally 1 m. in diameter, covered with pale smooth bark and short small branches forming a narrow round-topped head, and branchlets more or less thickly covered during their first season with white silky pubescence, usually gradually disappearing in their second year; in southern Florida often much smaller, and on the Everglade Keys, where it is "very common, a shrubby tree up to 3 m. high" (*E. A. Bessey*).

Swamps in the neighborhood of Wilmington, North Carolina, is the most northern station from which I have seen specimens of this tree; it is common in the coast region of South Carolina and Georgia and in all parts of Florida, and the only form of *M. virginiana* in the other Gulf states, where it occurs as far west as the valley of the Nueces River in Texas (San Augustine County), but is much less common west of the Mississippi River than it is farther east. Although it crosses the Florida peninsula this *Magnolia* is most abundant in the coast region. It ranges inland, however, to Cuthbert, Randolph County, in western Georgia, to Tuskegee and Selma, Alabama, and to Tishomingo County in the extreme northeastern corner of Mississippi, and to Winn and Natchitoches parishes in western Louisiana.

MAGNOLIA ACUMINATA var. **ludoviciana,** n. var.—Differing from the type in its broadly obovate, oval, or ovate leaves abruptly short-pointed at apex and rounded or cuneate at base, and in its much larger flowers. Leaves hoary tomentose below and slightly pubescent above when they unfold, becoming when the flowers open glabrous and yellow-green on the upper surface and pubescent on the lower surface with short pale hairs, 15–18 cm. long and 9–13 cm. wide; petioles puberulous, 2.5–4 cm. in length. Flowers 8–10 cm. long, the outer petals up to 4 cm. in width.

A large tree.

Rich woods, West Feliciana Parish, Louisiana, Dessert Plantation near Catalpa, *Cocks* and *Sargent*, April 12, 1916 (type); West Plantation near Catalpa, *Cocks* and *Sargent*, April 10, 1914; near St. Francisville, *R. S. Cocks*, May 15, 1915, and Catalpa, October 15, 1915.

ACER SACCHARUM Marsh.—The lower surface of the leaves of the northern sugar maple is usually green and glabrous, but it is sometimes glaucous or glaucescent and southward is slightly pubescent along the under side of the midribs and veins; and as the pale color of the lower surface of the leaves gives the trees a distinct appearance the varietal name adopted for them by some European dendrologists will probably be helpful. This form becomes

ACER SACCHARUM var. **glaucum,** nov. comb.—*A. saccharinum* var. *glaucum* Pax, Engler Bot. Jahrb. 7:242. 1886; Wesmael in Bull. Soc. Bot. Belg. 29:61. 1890.—*A. palmifolium* var. *glaucum* Schwerin, Gartenflora 42:455. 1893.—The leaves of this variety resemble those of the green-leaved variety in size and shape and are glabrous or in the southern states usually slightly pubescent on the under side of the midribs and veins.

From the North, where it is much less common than the green-leaved form, I have seen specimens of this variety only from Isle-aux-Couvres in the St. Lawrence River, from Prince Edward Island, Nova Scotia, Lake St. John and St. Anne's, Quebec, northern Vermont, Cooperstown, New York, western Pennsylvania, and Youngstown, Ohio; it is more common in southern Michigan and Indiana, and occurs in northeastern Iowa and central Tennessee. It is still more common in Missouri and northern Arkansas, and is the only form I have seen from South Carolina, Alabama, Mississippi, Louisiana, and southern Arkansas, where the sugar maple is not a common tree.

ACER SACCHARUM var. RUGELII Rehder, Cyclopedia Am. Hort. 1:13. 1900; Sargent, Man. *fig. 515.* 1905.—*A. Rugelii* Pax, Engler Bot. Jahrb. 7:243. 1886.—*A. saccharinum* subspec. *Rugelii* Wesmael, Bull. Soc. Bot. Belg. 29:61. 1890.—In *The Silva of North America* this form of the sugar maple was confused, at least in part, with *A. nigrum.* As it is now understood, the leaves of this variety are usually broader than long and are cordate or rounded at base, 3-lobed with long acuminate lobes, usually entire or the lower lobes occasionally furnished near the base with a small rounded lobe; the leaves are 3-nerved, thick, dark green above, green or glaucescent and glabrous on the lower surface, but on specimens collected by *Palmer* at Williamsville, Wayne County, Missouri (no. 6096), and from a large tree with short-lobed leaves at Campbell, Dunklin County, Missouri (*C. S. Sargent,* October 5, 1910), the lower surface of the leaves is thickly covered with loose pubescence.

This variety appears to be rare and local and to occupy a comparatively restricted area. The type station is at Dandridge on the Tennessee River, Jefferson County, Tennessee, and I have seen specimens from Knoxville, Tennessee, Eureka Springs, northwestern Arkansas, Williamsville, Campbell, and Allenton, Missouri, Lansing, Ingram County, Michigan, from Parry Sound, Georgian Bay (*B. E. Fernow*, 1908, a single tree), and Point Pelee in Lake Erie, Essex County, Ontario (*C. K. Dodge*, 1911).

ACER SACCHARUM var. **sinuosum**, n. var.—*A. sinuosum* Rehder, Sargent, Trees and Shrubs, **2**:255. *pl. 195*. 1913.—The distinctive character of *A. sinuosum* was found in a projection into the broad sinus at the base of the leaves formed by the nerves of the 2 upper lobes which form the base of the sinus. Since the species was described, large collections of this maple of the Edwards Plateau in western Texas show that this projection of the nerves is not a constant character and that *A. sinuosum* must be considered a small-leaved form of *A. saccharum*.

This little Texan tree is known only on the banks and bluffs of Cibelo Creek, near Boerne, Kendall County, on the rocky banks of the upper Seco Creek, Bandera County, and at the base of a high limestone bluff near Utopia, Uvalde County. Its isolation is remarkable and interesting, for none of the group of sugar maples grow nearer to the Edwards Plateau than *A. grandidentatum* Nuttall on the mountains in the extreme western part of Texas, *A. floridanum* Pax at Marshall, Harrison County, Texas, and *A. leucoderme* Small and *A. saccharum* var. *glaucum* Sargent in the Red River Valley in southern Arkansas.

ACER FLORIDANUM Pax.—*A. saccharinum* Elliott, Sk. **1**:450 (at least in part). 1821.

The range of this species can be extended northward from River Junction, Florida, which is the type station, through the Piedmont region of Georgia and the Carolinas to the banks of the Roanoke River, near Weldon, Halifax County, North Carolina, and to Dinwiddie County, southeastern Virginia (river banks and low wet woods near McKenney, *W. W. Ashe*). It is common in the neighborhood of Raleigh, Wake County, North Carolina, where it has been largely planted, and is the common and prevailing street tree.

Recent collections show that the variety of *A. floridanum* with villose-tomentose petioles and usually pubescent branchlets is not uncommon. It is the

Var. FILIPES Rehder, Sargent, Trees and Shrubs, **2**:255. 1913.—*A. brachypterum* Wooton and Stanley, Contrib. U.S. Nat. Herb. **16**: part 4, 146. 1913; **19**:411. 1915.

The type station of this variety is Columbus, Muscogee County, Georgia. It has also been collected in Georgia at Cuthbert, near Milledgeville, Mayfield, and on Shell Bluff on the Savannah River below Augusta, at River Junction, Florida, Calhoun Falls, South Carolina, in the streets of Raleigh, North Carolina, at Campbell, southeastern Missouri, and on the San Luis Mountains in southern New Mexico (*A. brachypterum*). This isolated New Mexican station far to the westward of the region usually occupied by *A. floridanum* is remarkable, but in the shape of the leaves, in their pubescence and in that of the petioles, pedicels, and branchlets, and in the length of the wings of the fruit, it appears identical with some of the specimens of the var. *filipes* from the southeastern states.

ACER RUBRUM Linn.—*A. carolinianum* Walter, Fl. Car. 251. 1888.—*A. stenocarpum* Britton in Britton and Shafer, N. Am. Trees 647, *fig. 598*. 1908.—The leaves of the red maple are usually green and glabrous or pubescent below early in the season, generally soon becoming glaucescent or glaucous below and glabrous or they are usually rather longer than broad, generally cordate or sometimes rounded at base, 3–5-lobed by acute sinuses with serrate lobes and slender glabrous petioles; in the autumn they turn scarlet on some trees and bright yellow on others. The flowers are red or yellowish green (var. *pallidiflorum* Pax). The fruit on different trees is red, yellow, or brownish. The branchlets and winter-buds are glabrous.

The red maple grows on the borders of streams, in wet swamps and in upland forests, occasionally on dry hills, and is found from Newfoundland to the banks of the Miami River in the extreme southern part of Florida, and westward to western Wisconsin, Minnesota, eastern Oklahoma, and to the neighborhood of Houston, Harris County, Texas.

A. stenocarpum Britton is based on a single small stunted tree growing on a dry hill of flint rock at Allenton, St. Louis County, Missouri, on which the samaras of the fruit vary in width up to 6 mm. This maple has been growing in the Arboretum for several years.

No other North American tree ranges through so many degrees of latitude as separate Newfoundland from southern Florida. In the shape of the leaves and in their pubescence, and in the size of the fruit, *A. rubrum* shows much variation. The extremes of these varieties have sometimes been considered species, but they are connected by so many intermediate forms that a better idea of the red maple can perhaps be obtained by treating it as a species with the following varieties:

ACER RUBRUM var. TOMENTOSUM Pax, Engler Bot. Jahrb. 7:182. 1886.—*A. Drummondii* Small, Fl. Southern U.S. 741 (insomuch as

relates to Georgia and Florida, not Hooker and Arnott). 1903.—This variety, which was based on trees cultivated in Europe, is distinguished by the close pale pubescence which covers the lower surface of the leaves during the season. The leaves are 5-lobed, cordate or rarely rounded at base, and the petioles are glabrous or slightly pubescent early in the season. The branchlets are usually glabrous and the winter-buds are pubescent.

I have seen specimens of this form of the red maple from Biltmore, North Carolina (Herb. Bilt. no. 116b), from the neighborhood of Augusta, Georgia, from the top of Flagstaff Mountain, Barclay, Alabama, Panther Burn, Sharkey County, Mississippi, Larissa, Cherokee County, Texas (*B. F. Bush*, May 1, 1909, no. 5579), near Page, Leflore County, Oklahoma (*G. W. Stevens*, no. 2617), and swamps near Little Rock, Pulaski County, Arkansas.

A specimen of this variety with pubescent branchlets and winter-buds, and slightly pubescent petioles, collected by *J. K. Small* at the Altamaha River Swamp, Liberty County, Georgia, in June 1895, and specimens collected by *Mohr* in April 1895 at Mount Vernon, Mobile County, Alabama, with broadly ovate, 3–5-lobed, slightly cordate leaves with pubescent petioles, fruit only 3.5 cm. long, and glabrous branchlets, serve to connect the variety *tomentosum* with

ACER RUBRUM var. DRUMMONDII Sarg.—The leaves of this tree are often broader than long, cordate at base, usually 5-lobed, with stouter midribs and veins than those of the other forms of *A. rubrum*. Until nearly fully grown the leaves are covered on the upper surface with scattered pale hairs and are clothed below with thick snow white tomentum which is more or less persistent during the season; the petioles are stouter than those of the other forms of the red maple, and are covered during the season with thick white tomentum similar to that on the under surface of the leaves. This gradually disappears and the petioles often become nearly glabrous in the autumn. The fruit, which ripens in early spring before or with the unfolding leaves, varies from 5 to 6 cm. in length.

This maple, which is a small tree usually not more than 10–12 m. high, inhabits deep river swamps often inundated through the year. It is distributed from the valley of the Hastchatchee River, Forrest County, southern Mississippi, through Louisiana to the valley of the Neches River, Texas (Beaumont and Concord). It is not rare in southern and eastern Arkansas, southeastern Missouri, and occurs in northwestern Mississippi (Morehead,

Sunflower County), and in southwestern Indiana (in a cypress swamp 18 miles west of Decker, Knox County, *C. C. Deam.*)

In the broad 3–5-lobed leaves cordate at base this maple is very distinct from other forms of *A. rubrum*, but trees occasionally occur with 3-lobed leaves rounded at base. This form may be described as

ACER RUBRUM var. DRUMMONDII f. **rotundata**, n. f.—Differing from the variety in the 3-lobed leaves rounded at base.

Specimens of this form have been collected in Louisiana at Chopin, Natchitoches Parish, *E. J. Palmer*, May 6, 1915 (no. 7553 type), and at Glen Gordon, Covington, St. Tammany Parish, *R. S. Cocks*, March 28, 1911; in Texas near Beaumont, Jefferson County, *C. S. Sargent*, April 11, 1915; and in Missouri at Poplar Bluff, Butler County, *G. W. Letterman*, September 3, 1882.

The fruit of this form has not been collected, but the tomentum of the leaves, petioles, and branchlets is that of the var. *Drummondii*. The shape of the leaves shows a transition into

ACER RUBRUM var. TRIDENS Wood.—*A. barbatum* Michaux, Fl. Bor. Am. 1:252 (at least insomuch as related to the leaves). 1803; Elliott, Sk. 1:451 (at least in part). 1821.—*A. carolinianum* Britton in Britton and Shafer, N. Am. Trees 648 (not Walter). 1813.—The leaves of this maple are obovate, narrowed from above the middle to the rounded or rarely cuneate base, 3-lobed at apex, coarsely serrate usually only above the middle, often ovate or oblong-ovate by the suppression of the lateral lobes, green or glaucous and glabrous, pubescent or tomentose on the lower surface. The flowers and fruit are red or yellow.

It has been found from Massachusetts to Florida, Missouri, and in eastern Texas to Harden and Cherokee counties, but is most abundant southward and sometimes, as in Richland Parish, northern Louisiana, it is the prevailing form.

The extreme forms of this variety are distinct, but the 3-lobed leaves often occur on trees with leaves of the normal form of the red maple, and the leaves on vigorous shoots of trees of this variety are often 5-lobed.

WALTER'S specimen of his *A. carolinianum* is preserved in the British Museum and is a typical *A. rubrum*, not the variety *tridens*. The leaves of *A. barbatum* as described by MICHAUX and ELLIOTT seem to be those of *A. rubrum* var. *tridens*. Their "pedunculi solito pilosi" might apply to *A. floridanum filipes* Rehder.

ACER NEGUNDO L.—The box elder or ash-leaved maple, which is one of the most widely distributed trees in the United States, has

assumed slightly different forms in different parts of the country. In what is considered the typical species, which occurs in the region east of the Rocky Mountains, the united part of the samaras of the fruit is more or less constricted at the base into a short stipe, and on the more western forms this constriction usually does not occur. This constriction and its absence, together with the absence or presence of pubescence on the leaves and branchlets, have sometimes been used to separate *A. Negundo* into several species, but the characters on which these species have been based are not particularly important, and it seems better to treat *A. Negundo* as a species with a number of varieties which often intergrade, for the characters on which they are based are not always constant.

In what is considered the typical species the branchlets are green and glabrous; the leaves are usually 3-foliate but occasionally 5–7-foliate; the leaflets are ovate to elliptic or oblong-obovate, acuminate and often long-pointed, rounded or cuneate and often unsymmetrical at base, coarsely and irregularly serrate usually only above the middle, and occasionally slightly lobed, slightly pubescent above and more or less tomentose below when they unfold, and at maturity glabrous above, usually villose-pubescent along the under side of the midribs and veins, and often furnished with conspicuous tufts of axillary hairs, otherwise glabrous or slightly pubescent below.

The typical form is distributed from western New England and central New York to Minnesota, Iowa, and Missouri, and southward to central Florida, northern Alabama, western Louisiana, and eastern Texas. I have not seen specimens of wild trees from eastern New England, eastern New York, New Jersey, or Delaware. The box elder is common along the St. Lawrence River near Montreal and in eastern Ontario, but these trees are believed to have been naturalized in recent years. As here considered it passes into the following varieties:

ACER NEGUNDO var. VIOLACEUM Kirchner in Kirchner and Petzold Arb. Mosc. 190. 1864.—*Rubac Nuttallii* Nieuwland, Am. Middl. Nat. 2:137. 1911.—*Negundo Nuttallii* Rydberg, Bull. Torr. Bot. Club 40:55. 1913.—This variety is distinguished by its rather stouter, pale or bluish violet branchlets covered with a glaucous bloom, rather larger buds, and usually 3–7-foliate leaves

with slightly thicker, lanceolate to oblong-ovate or obovate, often entire or irregularly dentate, occasionally lobed leaflets, the terminal leaflet occasionally 3-lobed, glabrous above and usually slightly pubescent over the lower surface; the base of the fruit is usually but not always constricted.

This variety is distributed from western Massachusetts, through New York to Ohio, northern Wisconsin, Minnesota, Iowa, South Dakota, Dufferin, Manitoba, and Nez Perce County, Idaho; it is common in northern Missouri and occurs near Noel, McDonald County, in the extreme southwestern part of that state (*E. J. Palmer*, no. 5479).

ACER NEGUNDO var. TEXANUM Pax, Engler Bot. Jahrb. 7:212. 1886.—*A. californicum* var. *texanum* Pax, *l.c.* 11:75. 1890.—*Rubac texana* Small, Fl. Southern U.S. 743 (in part).—*Negundo texanum* Rydberg, Bull. Torr. Bot. Club 40:56. 1913.—This variety is best distinguished by the 3-foliate leaves with broader ovate to obovate, coarsely serrate leaflets cuneate or rounded at base and covered below through the season with loose pubescence. The branchlets are pale pubescent or tomentose during their first season and the body of the fruit is usually puberulous and slightly or not at all constricted at the base.

This variety occurs in western (Jackson County) and southwestern Missouri, northeastern Kansas, through Arkansas to western Oklahoma and to the valley of the San Antonio River, Texas. It appears to have been collected first by *Lindheimer* near New Braunfels, Texas, in 1843 (no. 360 in Herb. Gray). Eastward it passes into

ACER NEGUNDO var. TEXANUM f. **latifolium,** n. f.—*A. Negundo* var. *latifolium* Pax, Engler Bot. Jahrb. 11:75. 1890.—Only differing from typical var. *texanum* in its glabrous branchlets and usually glabrous fruit often slightly constricted at the base.

This form occurs in eastern Texas, southern Arkansas, Louisiana, in the valley of the Black River, eastern Mississippi, at Nashville, Tennessee, on the banks of the Catawba River near Marion, North Carolina, in Virginia, and southern Ohio.

ACER NEGUNDO var. **interior,** n. var.—*A. interior* Britton in Britton and Shafer, N. Am. Trees, 655, *fig. 608.* 1908.—?*A. Kingii* Britton, *l.c.* 1908.—*Rulac interior* Nieuwland, Am. Mid. Nat. 2:139. 1911.—*Negundo interius* Rydberg, Bull. Torr. Bot. Club

40:56. 1913.—*Rulac texana* Small, Fl. Southern U.S. 743 (in part). 1903.—This variety, the box elder of the Rocky Mountains, differs from the variety *texanum* only in its narrower and usually more acuminate and more irregularly serrate, often lobed leaflets usually covered below with a closer pubescence, and in the more pubescent or tomentose petioles and rachis.

It ranges from Manitoba, Saskatchewan, and Alberta southward through Wyoming, Montana, Colorado, Utah, New Mexico, and Arizona. The oldest specimen of this tree which I have seen was collected by *A. Fendler* in New Mexico in 1847 (no. 102 in Herb. Gray).

More distinct is the variety of Arizona and southern New Mexico, which may be described as

ACER NEGUNDO var. **arizonicum,** n. var.—Leaves thin, 3-foliolate; petioles slender, glabrous, 4.5–7 cm. in length, often turning bright red late in summer; leaflets oblong-ovate to rhombic, acuminate and long-pointed at apex, rounded or cuneate at base, coarsely serrate, often slightly lobed near the middle, glabrous at maturity with the exception of conspicuous tufts of axillary hairs, 6–10 cm. long and 3–5 cm. wide; petiolules slender, glabrous, usually bright red, those of the terminal leaflet 2–2.5 cm. in length, the others only 6–7 mm. long. Flowers not seen. Racemes of fruit glabrous, 8–10 cm. in length; body of the fruit spreading, glabrous, not constricted at the base.

A tree 7–8 m. high with light gray fissured bark and slender glabrous branchlets thickly covered with a glaucous bloom.

ARIZONA.—Cave Creek Canyon, east slope Chiricahua Mountains, *J. W. Toumey*, July 1894; Oak Creek Canyon near Flagstaff, Coconino County, *A. Rehder*, July 14, 1914 (no. 34); Sycamore Canyon near Flagstaff, *Percival Lowell*, October 1915 and 1916; Santa Catalina Mountains, *J. G. Lemmon*, May 1881 (no. 128 in Herb. Gray); Mount Kellogg, Santa Catalina Mountains, altitude 2700 m., *A. Rehder*, August 31, 1916 (no. 463 type).

NEW MEXICO.—Kelley's Ranch, 7 miles north of Alma, Socorro County, *A. Rehder*, August 13, 1914 (nos. 279, 280; growing near no. 281, with densely pubescent branchlets and leaflets pubescent below, and so referable to var. *interior*); Glenwood, 7 miles south of Alma, Socorro County, *A. Rehder*, August 14, 1914 (no. 300).

This is the most glabrous of the forms of *A. Negundo*, and in its thin leaflets, bright red petioles, and glabrous branchlets thickly covered with a glaucous bloom one of the most distinct of them all.

ACER NEGUNDO var. CALIFORNICUM Sargent, Garden and Forest, 4:148. 1891; Silva N. Am. 2:112. *pl.* 97. 1891.—*Negundo californicum* Torrey and Gray, Fl. N. Am. 1:250, 684. 1838; Rydberg, Bull. Torr. Bot. Club 40:56. 1913.—*Acer californicum* Dietrich, Syn. 2:1283. 1840; Pax, Engler Bot. Jahrb. 7:213 (in part). 1836; 11:75. 1890.—*Negundo aceroides* Torrey, Pacific R.R. Rep. 4:74 (not Moench). 1857.—*Negundo aceroides* var. *californicum* Sargent, Garden and Forest 2:364. 1889.—*Acer Negundo* subsp. *californicum* Wesmael, Bull. Soc. Bot. Belg. 29:43. 1890.—*Rulac californica* Nieuwland, Am. Mid. Nat. 2:139 (in part). 1911.—Leaves trifoliate with tomentose or nearly glabrous petioles, rachis, and petiolules; leaflets oblong-ovate to rhombic, acuminate and long-pointed at apex, cuneate or unsymmetrically rounded at base, coarsely serrate above the middle or nearly entire, occasionally deeply lobed, glabrous on the upper surface except along the midribs and veins, thickly coated on the lower surface with matted pale hairs and furnished with large axillary tufts. Fruit on pubescent pedicels, puberulous or nearly glabrous, not constricted or rarely slightly constricted at base.

A large tree with dark bark, hoary tomentose branchlets, and winter-buds. Valley of the lower Sacramento River southward to San Bernardino County, California. The California box elder was discovered by DAVID DOUGLAS in 1833, probably in the neighborhood of Monterey.

FRAXINUS AMERICANA var. **subcoriacea,** n. var.—Differing from the type in its thicker, entire or slightly serrate leaflets, silvery white on the lower surface. Individual trees of *F. americana* occur with thick, oblong-ovate, acuminate, entire or slightly serrate leaflets dark green and lustrous above, silvery white below, glabrous or slightly villose along the midribs, and 7.5–13 cm. long. These trees are so distinct in appearance and in their more rapid and vigorous growth that it seems desirable to give them a varietal designation. What may be considered the type of the variety has been growing in the Arnold Arboretum since 1874, where it was raised from seed sent by *W. C. Hampton* from Mount Victory, Harding County, Ohio, as "*Fraxinus* C." The trees of this variety have grown more rapidly and are handsomer than any of the other American ashes in the collection. In 1910 and 1912 I collected the

same variety at Campbell, Dunklin County, Missouri, where I saw only a single individual. *F. americana* no. 4206, *A.* and *E. G. Heller*, Texarkana, Texas, September 1898, with entire and equally thick leaflets but not so pale below, is probably the same form.

CASTANEA ALNIFOLIA var. **floridana,** n. var.—Differing from the type in the glabrous, lustrous under surface of the mature leaves and in its arborescent habit.

In sandy soil with *Quercus myrtifolia* Willd. on the shores of St. Andrews Bay near Panama City, Bay County, Florida, *T. G. Harbison*, May 28, 1917 (no. 10, type), December 10, 1918 (nos. 13, 14); Dover, Screven County, Georgia, *T. G. Harbison*, May 13, 1913.

A tree occasionally 13–14 m. tall, or sometimes shrubby.

Unfortunately I have not seen the fruit of this tree, but in the shape of the leaves and in their serration, in the inflorescence, and in the glabrous branchlets it is not distinguishable from *C. alnifolia* Nutt. The leaves when they first unfold are hoary tomentose below, and the tomentum is sometimes persistent during the season on the upper leaves of vigorous shoots.

On a specimen of what appears to be the same form collected by *Harbison* near Jacksonville, Florida, the branchlets are slightly puberulous, and there are a few hairs on the under side of the midribs of the otherwise glabrous leaves.

The leaves of a shrub with pilose branchlets collected by *Harbison* on the coast near Wilmington, North Carolina, are broadly obovate and green, lustrous and puberulous on the under surface, and quite different from the leaves of the typical form of *C. alnifolia*, which are narrow-obovate to oblong-elliptic and thickly covered below with pale tomentum.

ARNOLD ARBORETUM
JAMAICA PLAIN, MASS.

POSSIBLE CORRELATIONS CONCERNING POSITION OF SEEDS IN THE POD

BYRON D. HALSTED[1]

This study was made with the Henderson Lima bean on a block of ground one-fortieth of an acre. Nine rows of 10 hills each were planted 5 seeds to the hill in the manner shown in table I.

TABLE I

Row	Position of seeds in pod	Percentage of viability
1	2-seeded base	52
2	2-seeded tip	70
3	3-seeded base	60
4	3-seeded middle	76
5	3-seeded tip	56
6	4-seeded base	64
7	4-seeded first middle	60
8	4-seeded second middle	72
9	4-seeded tip	74
Total		584

Table I shows that seeds from the middle of 3-seeded pods had the highest viability, and that of those from the base of 2-seeded pods nearly one-half failed to produce plants. The average viability of the 3 rows planted with basal seeds was 58.6 per cent, the next lowest 66.6 per cent in the rows planted with tip seeds, and the highest viability 69.3 per cent, obtained in the rows planted with seeds from the middle of 3-seeded and 4-seeded pods.

Table II shows that the pods are chiefly of the 3-ovuled type, and that the others are somewhat equally divided between the 2-ovuled and 4-ovuled pods. The yield of pods from seeds of 2-ovuled pods is 15.61 per cent less than the average yield from seeds of 3-seeded and 4-seeded pods.

Pods from plants grown from seeds borne in the basal position numbered 1463, from seeds from middle position 1735, and in the

[1] This paper was received from the late Professor HALSTED in January 1918. A brief biographical sketch appears in the BOTANICAL GAZETTE of February 1919.

tip position 1403. This is 23.66 per cent more pods from the 3 rows planted with seeds from the middle of the pod than from the same area planted with seeds from the tip position, and

TABLE II

NUMBER AND TYPES OF PODS FOR EACH ROW, BOTH HARVESTS COMBINED

Row	Position of seeds in pod	2-ovuled	3-ovuled	4-ovuled	Total	Average per pod type
1.....	2-seeded base	44	343	17	404	.....
2.....	2-seeded tip	37	449	21	507	455.5
3.....	3-seeded base	55	487	36	578	.....
4.....	3-seeded middle	82	540	34	656	523.0
5.....	3-seeded tip	30	288	17	335	.....
6.....	4-seeded base	44	401	36	481	.....
7.....	4-seeded first middle	16	517	46	579	530.2
8.....	4-seeded second middle	47	388	65	500	.....
9.....	4-seeded tip	60	456	45	561	.....
	Total.....	415	3869	317	4601	.....

18.58 per cent more than the corresponding rows planted with basal seeds.

The percentage of each pod type in the crop for each pod type planted is given in table III.

TABLE III

Type planted	2-ovuled	3-ovuled	4-ovuled
2-seeded pods.....	8.88	86.95	4.17
3-seeded pods.....	9.09	84.87	5.54
4-seeded pods.....	9.48	81.11	9.41
Total.....	9.30	83.87	7.33

Table III shows but little variation in the number of 2-ovuled pods, but in 3-ovuled pods there is a decided decrease in the percentage, going from 2-seeded to 4-seeded, and, of course, a corresponding increase in the 4-ovuled pods, and where the percentages are low the differences are great, over 125.66 per cent between the crop from 2-seeded pods and that from 4-seeded pods. In other words, there is seen to be a tendency for seeds from 4-seeded pods to reproduce the mother type of pod. The number of pods for each

harvest and the percentages for the 3 types of pods are indicated in table IV.

TABLE IV

	Number of pods	Percentage of each type of pod		
		2-ovuled	3-ovuled	4-ovuled
First harvest.....	2672	7.11	84.73	8.16
Second harvest.....	1829	11.86	82.87	5.27
Total.....	4501	9.30	83.37	7.33

Table IV shows that the first harvest comprised nearly three-fifths of the total crop. It is further shown that the first harvest has its 2-ovuled pods below the average and its 3-ovuled and 4-ovuled pods above the average, while in the second harvest the reverse is seen. In other words, as the season advanced the average number of seeds in the pods increased.

TABLE V

Number and average weight of seeds

Row	Position of seeds in pod	Pods without aborts		Pods with aborts		Both averaged	
		Number	Average weight	Number	Average weight	Number	Average weight
1.....	2-seeded base	479	0.404 gr.	433	0.434 gr.	456	0.419 gr.
2.....	2-seeded tip	683	0.376	529	0.404	606	0.390
3.....	3-seeded base	881	0.376	539	0.411	710	0.394
4.....	3-seeded middle	912	0.396	662	0.424	787	0.410
5.....	3-seeded tip	413	0.390	371	0.419	392	0.405
6.....	4-seeded base	617	0.392	512	0.420	565	0.406
7.....	4-seeded first middle	799	0.391	630	0.422	715	0.407
8.....	4-seeded second mid.	878	0.385	783	0.415	831	0.400
9.....	4-seeded tip	803	0.360	528	0.387	666	0.378

Table V shows that the largest number of seeds (4624) was taken from the rows planted with seeds from the middle of the pods, while the number of seeds planted from the basal and tip seeds were 3461 and 3377 respectively. The average weight of all seeds from each row approximates the general average, the only striking deviation being in the lightness of the seeds grown from seeds produced in the tip position of 4-seeded pods.

Table VI shows under pod average (seed-weight for the 3 types of pods) that the heaviest seeds are produced in 3-seeded pods and the lightest are found in 4-seeded pods. It is also shown that the seeds in the basal position are lighter than elsewhere in the type of pod considered, and that in the combined harvests the seed weights

TABLE VI

RELATION OF POSITION OF HARVESTED SEEDS IN POD TO WEIGHT: I. SEEDS FROM PODS WITHOUT ABORTS AND AVERAGE FOR ALL 9 ROWS

	2-base	2-tip	3-base	3-middle	3-tip	4-base	4-first	4-second	4-tip	All
Both harvests	0.338	0.366	0.362	0.392	0.412	0.305	0.341	0.341	0.345	0.385
Pod averages	0.352			0.391			0.333			
First harvest	0.314	0.345	0.344	0.375	0.388	0.268	0.319	0.313	0.326	0.360
Pod average	0.329			0.369			0.307			
Second harvest	0.379	0.402	0.399	0.442	0.459	0.361	0.374	0.384	0.375	0.426
Pod average	0.390			0.434			0.374			

make a continuous rising series from the base to the tip for all types of pods. There is an exception in the records for separate harvests, but it is among 4-seeded pods, a type not largely represented, and small deviations are here to be expected. It is noted that the first harvest yields seeds of lighter weight than does the second harvest, and with a single exception (4-seeded base) the difference applies to each position in the pod.

TABLE VII

II. SEEDS FROM PODS WITH ABORTS AND AVERAGE FOR ALL 9 ROWS

	2-base	2-tip	3-base	3-middle	3-tip	4-base	4-first	4-second	4-tip	All
Both harvests	0.404	0.448	0.377	0.403	0.439	0.329	0.361	0.375	0.388	0.414
Pod averages	0.440			0.418			0.373			
First harvest	0.324	0.388	0.344	0.368	0.401	0.271	0.338	0.354	0.374	0.377
Second harvest	0.442	0.473	0.413	0.438	0.476	0.423	0.433	0.403	0.428	0.453

Table VII shows from the pod averages that the weight of the seeds decreases from 2-ovuled pods to 4-ovuled pods, and that the average weight of the seeds in 3-ovuled pods is very close to the average for all seeds. The averages for both harvests show that the lightest seeds are produced in the basal position, and that there is in all types of pods a uniform increase from the weights in the basal position to the weights in the tip position. In the first harvest the seeds in each position in each type of pod are lighter than the seeds for the same position in the second harvest.

A comparison of the weights of seeds for each pod position in the combined harvests of pods without aborts (table III) with those with aborts (table IV) shows that the former are uniformly lighter, as seen in the following statement:

	First harvest	Second harvest	Both harvests
Pods without aborts.....	0.360 gr.	0.426 gr.	0.385 gr.
Pods with aborts.....	0.377	0.453	0.414

The pod averages for seed-weights (tables III, IV) show that the seeds of 3-ovuled pods without aborts are heavier than are the seeds (average weights) in the other two types of pods, while among pods with aborts the average seed weight is greatest in 2-ovuled pods. This is shown in the following figures, in which both harvests are combined:

	2-ovuled	3-ovuled	4-ovuled
Pods without aborts.....	0.352 gr.	0.391 gr.	0.333 gr.
Pods with aborts.....	0.440	0.418	0.373

From these averages it is seen that the differences are considerable, and it may be assumed that it is due to local environment within the pod. For example, in a 2-ovuled pod the average weight of the seeds is 25 per cent more when one ovule aborts, 6.93 per cent in 3-ovuled pods, and 12.01 per cent in 4-ovuled pods. It is noted that the differences are greatest in the extreme or, as they may be called, exceptional pods. The influence upon the remaining seeds when two or more ovules abort in the larger types of pods cannot be determined from the records.

Percentages of gains in weight of seeds associated with aborts over the weight of seeds from pods without aborts for each position in the pod are as follows:

	2-base	2-tip	3-base	3-middle	3-tip	4-base	4-first	4-second	4-tip	All
Pod averages	19.25	22.40	4.12	2.81	6.79	7.87	5.87	12.17	12.46	7.79
	20.96		4.57			9.59				

It is seen that the gain of weight of seeds for pods with aborts over corresponding seeds in pods without aborts is greatest in 2-ovuled pods and least in 3-ovuled pods. It is further shown that the weight is most augmented in the tip position in all types of pods, and least in the position next above the base in 3-ovuled and 4-ovuled pods.

TABLE VIII

PERCENTAGE OF ABORTIVENESS FOR EACH POSITION OF SEEDS IN POD

Position of seeds in pod	First harvest	Second harvest	Both
2-seeded base	17.05	26.7	21.81
2-seeded tip	17.30	24.9	21.10
3-seeded base	14.30	23.0	18.65
3-seeded middle	13.10	23.4	18.25
3-seeded tip	17.80	24.8	21.30
4-seeded base	20.0	23.0	21.50
4-seeded first middle	16.2	22.8	19.50
4-seeded second middle	16.0	22.8	19.40
4-seeded tip	17.6	27.1	22.35

Table VIII shows that the abortiveness for each of the 9 rows is less in the first than in the second harvest. When the two harvests are combined, the range is from 18.25 to 22.35 per cent. It is further deduced that the average for the middle seeds (19.05 per cent) is the lowest, while the tip seeds yielded the highest (21.58 per cent) average abortiveness. From the standpoint of percentage of good seeds per pod, the middle seeds are the best for good crop productions.

TABLE IX

PERCENTAGE OF ABORTIVENESS FOR EACH POD POSITION IN WHOLE CROP

	2-base	2-tip	3-base	3-middle	3-tip	4-base	4-first	4-second	4-tip	All
Both harvests	49.55	11.88	40.11	12.95	13.25	52.63	13.93	5.26	4.34	19.75
Pod averages	30.74		.....	19.02	.....	.....	19.04		.....	.....
First harvest	31.77	8.83	47.34	7.96	2.29	55.50	12.84	5.50	3.21	16.83
Pod averages	20.31		.....	15.77	.....	.....	19.26		.....	.....
Second harvest	62.99	14.17	51.28	13.74	4.48	46.66	16.99	4.76	6.66	24.12
Pod averages	38.58		.....	23.17	.....	.....	18.57		.....	.....

Table IX shows that when the two harvests are combined the abortiveness is chiefly in the lower portion of the pod and is very large in the basal position. In all types of pods there is a regular decrease in the number of aborts from the base to the tip, with the

greatest range in 4-ovuled pods. The averages are more nearly alike than averages for position in the pod and are not strictly comparable.

In the first harvest the aborts are nearly two-thirds the number of those in the second harvest, but their distribution among the 9 positions in the pods does not follow fully the rule given for the whole crop. This is shown in the pod averages, where in the first harvest the smallest average is with the 3-ovuled pods, while in the second crop the smallest average is with the 4-ovuled pods.

A greater abortiveness in the second crop may be ascribed to the advanced age of the plants or to the lack of proper insect visitation, but this latter circumstance may not be significant, as Lima beans are understood to be self-fertilized. It is possible, of course, that the cause may be related to the atmospheric conditions prevailing at the time the ovules were ready to set, and this suggests the importance of repeating the present test through a series of years.

TABLE X

AVERAGE WEIGHT OF SEEDS AND PERCENTAGE OF ABORTIVENESS FOR EACH POD POSITION

	2-base	2-tip	3-base	3-middle	3-tip	4-base	4-first	4-second	4-tip	All
Seed weight ..	0.338	0.366	0.362	0.392	0.412	0.303	0.341	0.341	0.345	0.385
Abortiveness.....	49.55	11.85	40.11	12.95	3.25	52.63	13.93	5.26	4.34	19.75
Seed weight ..	7.0	3.0	4.0	2.0	1.0	8.0	6.0	6.0	5.0	.....
Abortiveness.....	2.0	6.0	3.0	5.0	9.0	1.0	4.0	7.0	8.0	.....

The ranking figures make it easier to compare the relationship of the average weights and abortiveness among their respective units. Table X shows at once that the position yielding the heaviest seeds has the lowest percentage of abortiveness, and, contrariwise, the position with the lightest seeds has the largest number of aborts. It is evident that the type of pod has much influence upon the weight of the seed, and it is only proper that the comparison here made should be within the pod. With this consideration in mind it is seen that the order from base to tip with all the pods is reversed, that is, the base bears the lighter (or lightest) seeds and with more (or most) abortiveness.

On account of the influence of pod type upon seed-weight and abortiveness it follows that the relative seed-weight does not necessarily determine the amount of abortiveness. For example, 2-ovuled pods bear seeds at their tips that are of the same weight as those formed at the base of 3-seeded pods, but the relative abortiveness is 11:40.

Summary

1. The greatest viability in Henderson Lima beans is associated with the seeds that are borne in the middle of the pods.

2. Three-seeded pods make up more than four-fifths of the crop; 3-seeded and 4-seeded pods are more numerous in the second of the two field harvests of ripe pods.

3. Seeds from the middle of the pod produce a much larger number of pods than do seeds from the base or tip.

4. The heaviest seeds are produced in 3-seeded pods, and the lightest in 4-seeded pods.

5. The seed weights make a continuous rising series from the base to the tip for all types of pods.

6. The first harvest yields lighter seeds than does the second harvest in each pod position.

7. The seeds associated with aborts are heavier than are those in full pods in each type of pod, and each position in the pod.

8. The abortiveness is less in the first than in the second harvest, and is least in the rows grown from seeds from the middle of the pods.

9. Abortiveness is chiefly in the basal position and decreases regularly from the base to the tip of the pod.

10. The position of the pod that yields the greatest weight of seed is associated with the lowest percentage of abortiveness.

EMBRYO AND SEEDLING OF DIOON SPINULOSUM

SISTER HELEN ANGELA DORETY

(WITH PLATES X, XI)

Dioon spinulosum Dyer, imperfectly and incompletely described by EICHLER[1] in 1883, and by DYER[2] in 1885, but fully and carefully by CHAMBERLAIN[3] in 1909, is, like the other 2 species of *Dioon*, endemic in Mexico. The embryos and seedlings which furnish the material for this study were grown from ovules collected by Dr. CHAMBERLAIN in the mountains about Tierra Blanca and Tuxtepec during his several trips to the *Dioon* country. The tree is described by him as a magnificent ornamental cycad 30–40 ft. high. Unlike *D. edule*, it grows rapidly, and in 2 years makes a handsome greenhouse plant with a crown of large, fernlike leaves.

The unique appearance of the plant and the great size of its ovulate strobilus and ovules led to the expectation of great peculiarities in its vascular anatomy and cotyledonary arrangements. The investigation has verified these expectations only in part. The study of the vascular anatomy of the embryo and seedling of *D. spinulosum* merely serves to emphasize the general harmony which prevails among the cycads in this respect.

Embryo

The seed, like those of all cycads, is filled with a massive endosperm stored with starch. Upon this tissue the proembryo and embryo proper are nourished, apparently without any resting period. When the embryo has attained a length equal to that of the seed itself, pressure is exerted upon the stony coat, and the thin region near the micropyle is broken. Fig. 1 shows a seed with a young embryo borne on the twisted suspensor; fig. 2 represents

[1] EICHLER, A. W., Ein neues *Dioon*. Gartenflora 2:411. 1883.

[2] DYER, SIR W. T. THISTLETON, Biologia Centrali Americana, Botany 3:190. 1885.

[3] CHAMBERLAIN, C. J., *Dioon spinulosum*. BOT. GAZ. 48:401–413. 1909.

a later stage in which the embryo has attained its full length, has broken the seed coat, and pushed out the dried remains of the suspensor and archegonial wall.

The embryo at this stage consists of cotyledons, plumule, and a basal part which in its upper portion is hypocotyl, and in its lower portion root sheath; for the root is endogenous, and is not preceded by any structure which might be looked upon as a "radicle" or "caulicle." The hypocotyl is extremely short, and the distinction between it and the root sheath cannot be determined superficially, but only by study of the internal structure. The number of cotyledons varies from 2 to 4, and all stages of their union are represented (figs. 20–22, 24).

The vascular cylinder of the hypocotyl is a protostele. It has 4 easily recognized protoxylem groups, in no way differing from the hypocotyl cylinders of *Ceratozamia*[4] and *Microcycas*.[5] The cotyledons are multifascicular, like those of *Ceratozamia* and *Microcycas*, and unlike those of *Zamia* and *Dioon edule*. In the embryos which are dicotyledonous, the manner in which the cotyledonary traces are supplied from the hypocotyl cylinder is exactly the same as that described by THIESSEN[6] for *Dioon edule*, and which COULTER and CHAMBERLAIN[7] have shown to be characteristic of the cycads. When the embryo has 4 cotyledons, each cotyledon is on a side of the quadrangular node, and receives a secondary bundle from each of the adjacent angles. Twelve out of 100 embryos had 4 cotyledons, and in each case the vascular strands arose in this manner. In the tricotyledonous embryos (there were 4 of them in 100), one of the 3 cotyledons was supplied in the dicotyledonary manner, and the other two after the manner of the embryos with 4 cotyledons; and yet, at a level just above the tip of the plumule, the number of vascular strands was about equal in the 3 cotyledons. The strands

[4] DORETY, HELEN A., The seedling of *Ceratozamia*. BOT. GAZ. 46:203–220. *pls. 12–16*. 1908.

[5] ———, Vascular anatomy of the seedling of *Microcycas calocoma*. BOT. GAZ. 47:139–147. *pls. 5, 6*. 1909.

[6] THIESSEN, REINHARDT, The vascular anatomy of the seedling of *Dioon edule*. BOT. GAZ. 46:357–380. *pls. 23–29*. 1908.

[7] COULTER, J. M., and CHAMBERLAIN, C. J., Morphology of gymnosperms. Chicago. 1910.

are endarch where they separate from the hypocotyl cylinder (fig. 28); they become mesarch just after they enter the base of the cotyledon (fig. 27) and maintain that character throughout the greater length of the organ; near its tip, however, they are exarch (fig. 26). In all cases the orientation is collateral ectophloic, although at levels where branching is effected there is always an apparent concentric arrangement where the 2 xylem masses are still in contact, and the phloem masses are swung to right and left of them. Transfusion tissue accompanies the metaxylem. Mucilage ducts and tannin cells are abundant.

The root meristem is plainly visible below the hypocotyl plate in embryos of the age shown in fig. 2, but no differentiation has taken place, and of course there is no vascular tissue. The 4 poles of the root are later developed in connection with the 4 protoxylem groups of the hypocotyl vascular plate.

The plumule consists of 3 or 4 abortive scales inclosing the rudiments of the first and second true leaves, sometimes of a third leaf, and the stem tip. There is no means of distinguishing between the stem tip rudiments and those of a new leaf, because the leaf meristem grows much more rapidly, and soon overtops the stem tip (fig. 14). The vascular system supplying all these bracts and leaves is complicated by the well known habit of girdling, the details of which have fascinated and baffled many investigators. Although *D. spinulosum* differs in no way from the other cycads in this respect, its greater size makes a naked eye drawing possible and thus furnishes a means for solution and demonstration. Figs. 14 and 15 were drawn from macerated stems. Each node of the stem is, like the nodes on a first year stem of foxglove, telescoped within the older one instead of growing above it. The internodes are not elongated because the primary meristem of the stem tip is held in check by the more rapidly growing secondary meristem for each developing leaf. Since each leaf is supplied with strands from cauline bundles in different parts of the stem, those strands which come to it from the opposite side of the stem describe almost a semicircle to reach the leaf; those which arise on the same side as the leaf pass directly into it; and small arcs are described by those strands which arise in intermediate positions. In fig. 14

the traces for the older leaf (1^1) are supplied from the cauline strands numbered 2, 7, 14, 17, and 20. The strands from 2 and 20 will girdle the stem cylinder, those from 7 and 17 will make a partial girdle, and that from 14 will enter the leaf petiole directly. Fig. 15 is an attempt at demonstrating the same condition lower down in the stem. For the purpose of making the condition clearer the vertical magnification was made greater than the horizontal one.

Many angiosperms which have both "radical" and cauline leaves give illustration of this same condition. In the second year stems of such plants each node is located at some distance above the older one, and the leaf traces arising on the side of the stem opposite the leaf describe a spiral or oblique arc before entering the leaf. In the part of the stem from which the so-called "radical" leaves spring, however, the vascular strands destined for these leaves describe a horizontal arc similar to the leaf trace girdle in all parts of the cycad stem.

A careful but vain search was made for cortical cambium, vestigial traces of the primitive polystele of the Cycadofilicales.

Germination

Germination is hypogean, like that of all other members of the order thus far described. When the embryo has grown to the full length of the seed, the thin portion of the stony coat surrounding the micropyle yields to the pressure exerted upon it by the base of the axis. This base, scarcely more than a root sheath, emerges, pushing before it the brown and withered remains of the suspensor and archegonial wall. The cotyledonary base elongates and bends downward, and the root tip emerges from its sheath (fig. 3). If the embryo is a monocotyledonous one, that is, if its whole cotyledonary apparatus is a single sheath surrounding the plumule, this sheath is split by the radial growth of the axis; if there are two or more distinct cotyledons, their petioles are separated by the same cause (fig. 4). The plumule then emerges from the seed and becomes erect. Of course, when the seeds germinate while in a vertical position, the root and the plumule develop in the same axis (figs. 5, 6). In 3 of the seeds double embryos developed (fig. 29).

Seedling

The primary root persists indefinitely as a tap root. Large quantities of starch are transferred to it through the cotyledons, and it becomes large and swollen. The small lateral roots arise in whorls, usually of 4, and become a matted mass of fibers. Almost all the greenhouse grown seedlings have their roots hypertrophied, and the root tips have the characteristic tubercles described by LIFE[8] in *Cycas*.

The first leaves are yellowish scales, although thick and fleshy. They bear stipules like those of the foliage leaves, and some of them manifest the typical circinate venation (figs. 10, 11). The true leaves have been described by CHAMBERLAIN in the work previously cited. The first true leaves of several of my seedlings had as few as 6 pairs of leaflets (fig. 9), but most of them had 12 or more. There is great variation in the size of leaves of plants grown in the same conditions and from the same sized seeds. One plant bore small leaves with leaflets 2×0.3 cm., while another just beside it bore on its first leaf 12 pairs of leaflets, each one of them 6×1.1 cm. Imitation of the moist air conditions under which the first leaves of *Ceratozamia* became foliage leaves was unsuccessful with *Dioon spinulosum*.

The stele of the primary root is tetrarch, changing to diarch in its later formed portions and in the lateral branches. The relative size of the vascular cylinder to the whole root in various levels is shown by figs. 16–19. In the hypocotyl the diameter of the vascular cylinder is only about one-seventh that of the whole axis. The manner in which the radial position is achieved in the root is illustrated in figs. 18 and 19.

The leaf traces are always endarch and collateral, and their arrangement in the petiole as shown in cross-section presents the well known omega-shape. Branching and anastomosis are frequent throughout the petiole, but there is in general a diminution of traces toward the top of the leaf.

The plant is a much more rapid grower than *Dioon edule*, and is far more graceful. Under favorable conditions in the greenhouse,

[8] LIFE, A. C., The tuber-like rootlets of *Cycas revoluta*. BOT. GAZ. 31:265–271. 1901.

plants made 3 and 4 leaves, each 1 m. long and 14 cm. wide, in less than a year after emerging from seed.

Summary

1. The cotyledons of *Dioon spinulosum* vary in number from 2 to 4, and they are often lobed and divided so as to appear greater in number. In rare cases the cotyledonary sheath is undivided except near the tip.

2. They are multifascicular, like those of *Ceratozamia* and *Microcycas*, rather than like those of *Zamia* and *Cycas*, which have but few strands.

3. The arrangement and orientation of the vascular strands of cotyledons, hypocotyl, stem, leaves, and root do not differ in any marked degree from the general cycad arrangement.

4. The stem is large enough to demonstrate the cause of the girdling habit and to bring it into alignment with certain angiosperms of the same habit.

5. There is no extrafascicular cambium or any other vestige of polystyle.

Grateful acknowledgment is due to Dr. CHARLES J. CHAMBERLAIN for the generous supply of material from which this study was made.

COLLEGE OF ST. ELIZABETH
CONVENT, NEW JERSEY

EXPLANATION OF PLATES X, XI

The drawings 1–13 and 29 were made with the unaided eye and are reduced to one-half the natural size; 14–19 are diagrammatic; the remainder were made with the aid of the Abbe camera lucida. The following abbreviations have been used: *c*, cotyledon; *l*, leaf; *s*, suspensor; *sc*, scale leaf; *st*, stipule; *vc*, vascular cylinder; *x*, xylem; *ph*, phloem; *px*, protoxylem; *mx*, metaxylem; *ep*, inner epidermis of the cotyledons.

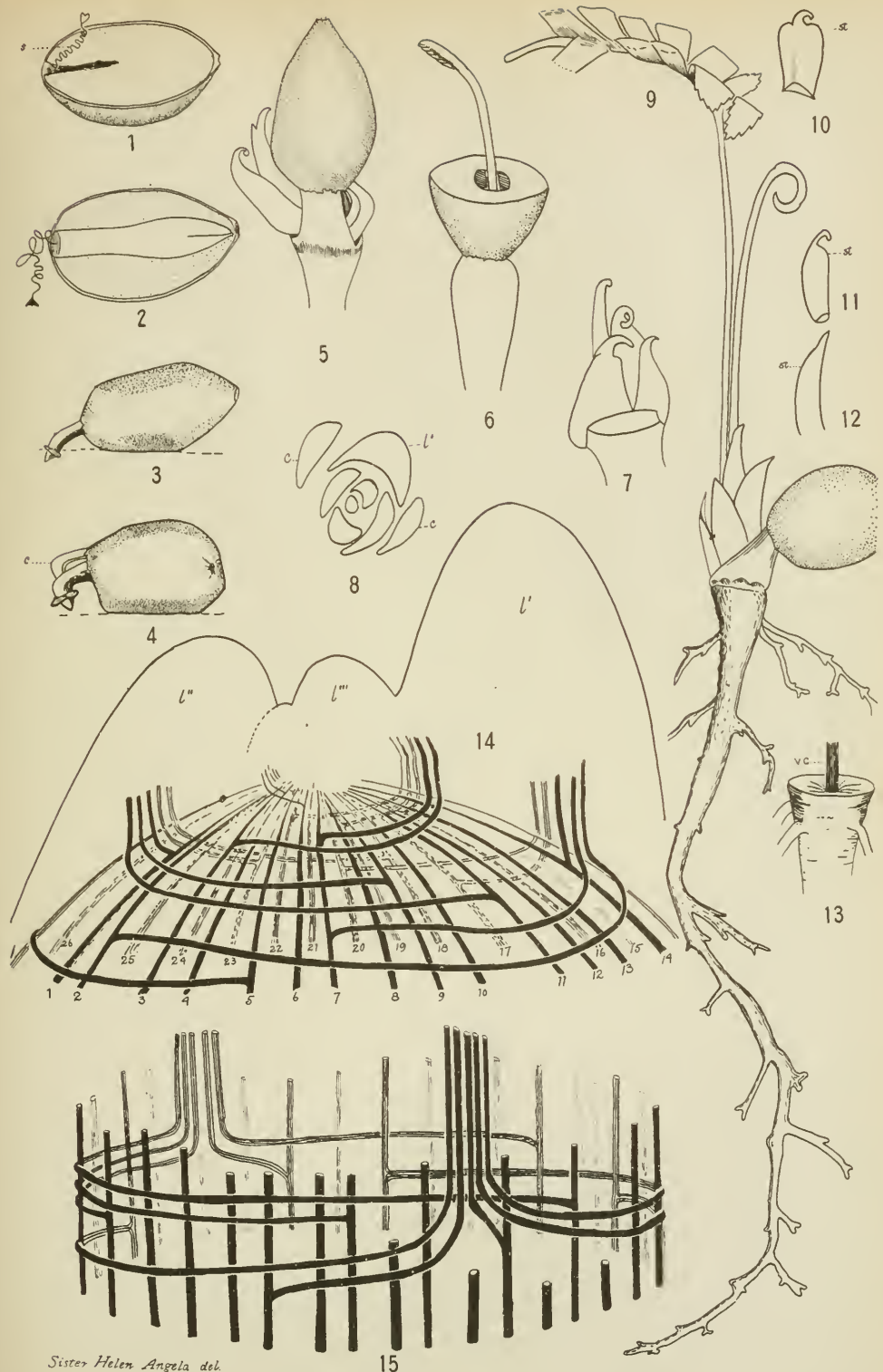
FIG. 1.—Ovule with young embryo and coiled suspensor.

FIG. 2.—Seed with mature embryo, pushing out suspensor and archegonial wall.

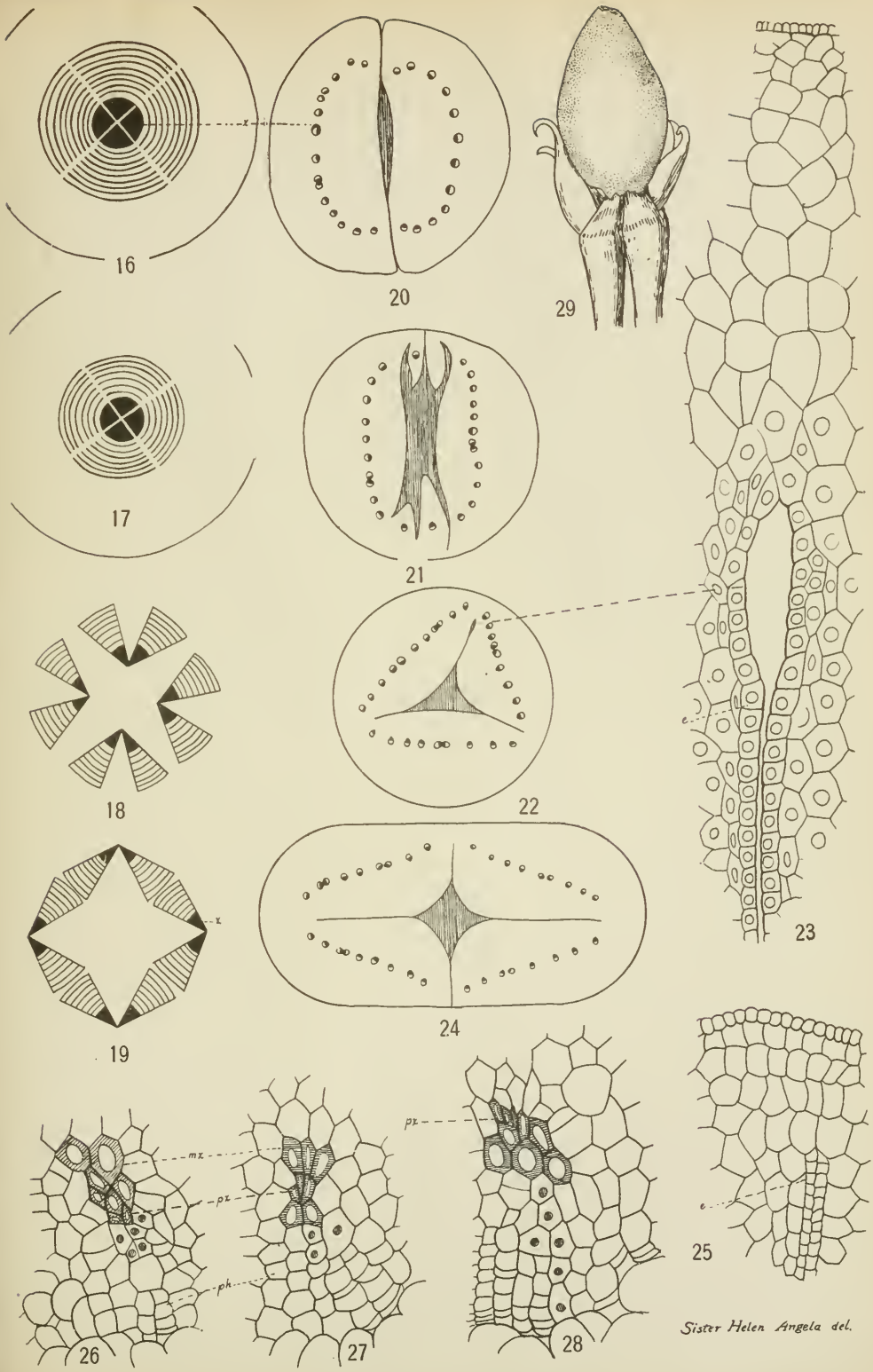
FIG. 3.—Beginning of germination.

FIG. 4.—Separation of cotyledonary petioles.

FIG. 5.—Seedling germinated in vertical position.



DORETY on DIOON


x
16
20
29
17
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18
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19
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mx
pz
ph
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26
27
28
Sister Helen Angela del.

FIG. 6.—Germination of lower half of seed.

FIG. 7.—First scale leaves, side view.

FIG. 8.—Cross-section of cotyledons and plumule, showing approximately opposite arrangement of leaves.

FIG. 9.—Seedling 2 months after germination.

FIGS. 10–12.—First, second, and third scales respectively.

FIG. 13.—Size of vascular cylinder to relative root.

FIG. 14.—Diagram showing cause of girdling of leaf traces and apparent absence of stem tip.

FIG. 15.—Girdling of leaf traces lower down in stem; vertical magnification greater than horizontal.

FIG. 16.—Diagram of hypocotyl cylinder or "plate."

FIG. 17.—Section 1 mm. below fig. 16.

FIG. 18.—Transition to root arrangement; separation of phloem masses of 4 poles.

FIG. 19.—Vascular cylinder of root.

FIGS. 20–22, 24.—Cotyledonary variations.

FIGS. 23, 25.—Details of arrangement of epidermis over inner sides of cotyledons and cotyledonary sheath.

FIG. 26.—Exarch bundle from tip of cotyledon.

FIG. 27.—Mesarch bundle from central part of cotyledon.

FIG. 28.—Endarch bundle from base of cotyledon.

DEVELOPMENT OF STROPHARIA EPIMYCES

W. B. McDOUGALL

(WITH TEN FIGURES)

Stropharia epimyces (Peck) Atk. was first described by PECK (9) in 1884 as *Panaeolus epimyces*. It was redescribed by ATKINSON (1) in 1902 and again in 1907 (2), and placed in the genus *Stropharia* because of the purplish tinge of the spores and the presence of an annulus. It is considered rare, but it has occurred in several localities north of Urbana during each of the 4 summers I have spent in Illinois, and was particularly abundant during the seasons of 1915 and 1916.

As is well known, this plant always occurs as a parasite on another mushroom. The identification of the host plant was first published by ATKINSON (1) as *Coprinus atramentarius*. Later a second host, *C. comatus*, was added by SHERMAN (10). All specimens collected at Urbana have been on *C. comatus*. Several photographs of *Stropharia epimyces* and its host were published by McDOUGALL (8), and excellent photographs were also published by ATKINSON (2).

Material for the developmental study of this plant was obtained within the city park of Urbana in Spetember 1915. It was imbedded, sectioned, and stained with fuchsin.

Development

The smallest carpophore sectioned measured 0.9 mm. by 1.2 mm. (fig. 1). In this the pileus and stem fundaments cannot be said to be differentiated, but the primordium of the hymenophore already appears as a patch of heavily stained hyphae on each side of the median longitudinal section. Aside from this hymenophore primordium there is no differentiation of the carpophore at this stage, except a layer of coarse and rather loose hyphae on the periphery, representing the universal veil or blematogen. The size of the carpophore is not always an index of its degree of

development, since the carpophore shown in fig. 2 is considerably larger, although little if any further developed than that shown in fig. 1; but in any case the first internal differentiation is the appearance of the hymenophore primordium.

The rapid growth of the elements of the hymenophore as the carpophore enlarges, together with the cessation of growth, or at least very slow growth of the ground tissue below the hymenophore primordium, soon cause the formation of an annular gill cavity (figs. 3, 4). The presence of the annular gill cavity makes it easy to see in longitudinal section which parts of the carpophore

FIG. 1

FIG. 2

FIGS. 1, 2.—*Stropharia epimyces:* fig. 1, small carpophore showing primordium of hymenophore as only differentiation; fig. 2, larger specimen at same stage of development.

in general belong to the pileus fundament and which to the stem fundament, but it is not until still later that these are clearly distinguished.

The gill cavity enlarges rapidly (fig. 5), but it does not become very large before the formation of the lamellae by the downward growth of hyphae from the hymenophore begins (figs. 6, 7). By this time also, if not earlier, the universal veil has disappeared, and the mature carpophore is without any trace of it. ATKINSON (4) has explained in detail the development of the lamellae in

Agaricus Rodmani. The development in our plant is similar and therefore the details need not be repeated here. The same author

FIG. 3

FIG. 4

FIGS. 3, 4.—*Stropharia epimyces:* stages in development of annular gill cavity.

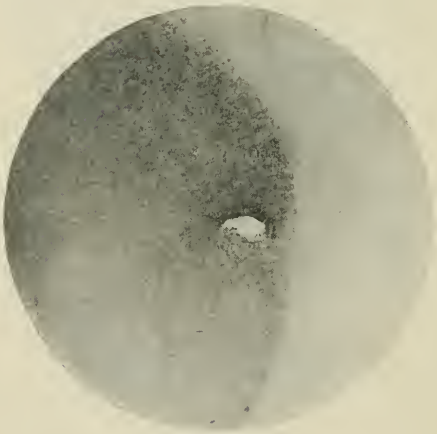

FIG. 5

FIG. 6

FIGS. 5, 6.—*Stropharia epimyces:* fig. 5, late stage in development of annular gill cavity; fig. 6, early stage in development of lamellae.

has explained the rather deceptive "stalls" or "pigeon holes" formed by the lamellae at the stem end in longitudinal sections of carpophores in which the lamellae are attached to the stem.

Such "stalls" are shown in figs. 8 and 9, and prove that the lamellae are attached to the stem at this stage of development.

FIG. 7

FIG. 8

FIGS. 7, 8.—*Stropharia epimyces:* fig. 7, late stage in development of lamellae; fig. 8, similar stage cut to show "stalls," indicating that gills are attached to stem.

FIG. 9

FIG. 10

FIGS. 9, 10.—*Stropharia epimyces:* fig. 9, portion of expanding carpophore showing "stalls," indicating that gills are attached to stem; fig. 10, portion of expanding carpophore showing the rather slight inner veil.

The inner veil which develops from the ground tissue below the hymenophore primordium remains intact until the lamellae are

well developed, but it usually ruptures before the stem is much elongated (fig. 10) and forms a rather slight ring on the stem. The subsequent elongation of the stem results in the position of the ring near the stem base.

Discussion

As ATKINSON (5) has said, the few species of Agaricaceae with endogenous origin of the hymenophore, whose development has been studied, fall into 3 groups, based on the order of differentiation of the pileus, stem, and hymenophore fundaments in the carpophore. In the first of these groups the pileus primordium is differentiated first. This group is represented by *Hypholoma sublateritium*, *H. fasciculare*, *Amanita rubescens*, and *Amanitopsis vaginata*. A second group includes those species in which the stem primordium is first differentiated, this being followed by the differentiation of the pileus primordium and later of the hymenophore primordium. This group is represented by *Lepiota cristata*, *L. seminuda*, and *Rozites gongylophora*. In the third group the hymenophore primordium appears while the remainder of the carpophore is seemingly undifferentiated, and the distinction of pileus and stem comes later. In this group we find *Agaricus campestris*, *A. arvensis*, *A. Rodmani*, *Armillaria mellea*, and *Stropharia ambigua*.

Stropharia epimyces, from its mode of development, is to be placed in the third group mentioned. This may be taken as additional evidence, if any further evidence is needed, that our plant belongs to the genus *Stropharia* and not to *Panaeolus*, as was first thought by PECK, since the type of development is the same as that of the only other species of *Stropharia* that has been studied (11) and of species of the closely related genus *Agaricus*.

Stropharia epimyces as it occurs at Urbana agrees in detail with the description given by ATKINSON (1, 2), except that it occurs on *Coprinus comatus* instead of *C. atramentarius* as did those collected by ATKINSON. The recognition of the host plant was easy in this case since there were many uninfected plants growing along with the infected ones, and also many plants which had "pinhead" and "button" stages of the parasite on them but were not deformed to such an extent as to be unrecognizable. The spores are distinctly purple-black in color. The annulus is often not very prom-

inent, but is usually perfectly apparent. The lamellae in mature specimens are adnate to adnexed.

HARPER (6, 7) has suggested that *Stropharia epimyces* is identical with *Pilosace algeriensis* (Fries) Quel. While it is entirely possible that this may be true, our plant cannot belong to *Pilosace*, as we understand that genus, since it has an annulus and the lamellae are not free.

The fact that all species of *Agaricus* and *Stropharia* thus far studied (*Agaricus comtulus*, see ATKINSON, 3, is a possible exception) develop in the same way is of interest as indicating a close relationship between these two genera. The main taxonomic characters that have been used to distinguish these genera are the free gills in *Agaricus* and the attached gills in *Stropharia*. ATKINSON (4), however, has found that in developmental stages of *Agaricus Rodmani* the gills are often attached, and that even in mature specimens of *A. Rodmani* and *A. campestris* the gills are sometimes adnexed, thus indicating that such characters do not clearly distinguish the genera.

UNIVERSITY OF ILLINOIS
URBANA, ILL.

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BRIEFER ARTICLES

HYBRID PERENNIAL SUNFLOWERS

From time to time references have been made to supposed hybrids between the perennial sunflowers, but there has been no systematic investigation of the subject. Such hybrids, if formed, might in many cases reproduce vegetatively, and so give rise to an essentially uniform group of plants of considerable extent, having the aspect of a true species.

At Boulder, Colorado, *Helianthus orgyalis* and *H. Maximiliani* have been growing in close proximity for a number of years. There has appeared close to these plants a distinct form which can hardly be anything but a hybrid between the two. Possibly such hybrids will be found growing wild in Nebraska, Missouri, or Texas, if anywhere the ranges of the parent species overlap. In order to bring out the characters of the new plant it is necessary partly to redescribe the supposed parents, especially since the descriptions in the manuals omit several significant characters. The 3 plants involved will be distinguished by the following numbers: (1) *H. orgyalis* DC.; (2) *H. orgyaloides*, nov. (the presumed hybrid); (3) *H. Maximiliani* Schrad.

Stems: (1) very smooth and glaucous to top, much branched, the branches slender; (2) essentially smooth, but roughish to the touch above, nearly as stout as in *Maximiliani*, and with few branches or short peduncles as in *Maximiliani;* (3) stout, little branched, scurfy, with matted white hairs, thinly hairy at top.

Leaves: (1) linear, crowded on stem, 1-nerved, but with a strong marginal nervure; surface glabrous; margins slightly undulate, with mere traces of obsolete teeth; width of stem leaf 6 mm.; (2) linear, appearing as in *orgyalis*, but up to 12 mm. broad, rough to the touch, remotely and indistinctly subdentate; a continuous but looped submarginal nervure; (3) broadened, narrow lanceolate, grayish, more or less scabrous on both sides, margins remotely and feebly dentate; no continuous marginal nervure; width of stem leaf 26 mm.

Peduncles: (1) slender; (2) stoutish; (3) stout.

Disk: (1) dark; (2) yellow, pale green in bud; (3) yellow.

Phyllaries: (1) linear, long, and spreading; (2) long, narrow, and spreading, of the *orgyalis* type; (3) lanceolate, loose, and spreading.

Rays: (1, 2, 3,) without pistils; (1) 11–14, rather more decidedly orange than in *Maximiliani*, and more or less bifid at end; (2) 14–21, clear bright orange-yellow, essentially *Maximiliani* color, many with tips deeply bifid; (3) 30, light yellow, more or less emarginate at end, and largely in 2 rows.

Diameter of disk: (1) about 10 mm.; (2) about 16 mm.; (3) about 17 mm.

Disk bracts: (1) very hairy at end, covering disk buds at early stage; no produced naked tips; (2) very hairy at end, covering disk buds at early stage, and with short naked tips; (3) hairy at end, covering disk buds at early stage, their tips elongate and sharp, pale green, not hairy.

Stigmatic branches: (1, 2, 3) orange.

Achenes: (1, 2, 3) entirely glabrous.

Pappus scales: (1) 2, about or hardly half length of corollas; (2) 2, about as long as in *orgyalis*, sometimes with well defined intermediate squamellae; (3) over half length of corollas.

The hybrid is on the whole intermediate. It is surprising that the dark disk is not dominant. A remarkable feature is the deeply bifid ends of the rays in the hybrid, greatly exaggerating the character of the parents. The appearance of intermediate pappus squamellae is a common feature in true *orgyalis*. Essentially this hybrid is evidently known to the trade, although not described. We have a purchased plant belonging to it, but differing from the one just described in the following particulars: stems freely branching as in *orgyalis*, and plant as tall as *orgyalis*;[1] disk olive green in bud; peduncles rather slender; rays 18–24, only slightly emarginate at end; disk about 15 mm. in diameter; lobes of disk corollas light orange, the extreme tips reddened, or whole lobe suffused with red. F. C. Heinemann, of Erfurt, Germany, advertised a supposed hybrid of this group, calling it *H. perennis hybridus pyramidalis;* but from his figure it seems to be simply *H. Maximiliani.*

It is clear that our hybrid does not agree with *H. Dalyi* Britton or *H. Kellermani* Britton. Dr. J. C. Arthur writes me that some years ago, near Madison, Wisconsin, he saw a considerable growth of *H. Kellermani*, and near by *H. grosseserratus* and *H. orgyalis*, or what appeared to be such. He had the idea that *H. Kellermani* was a hybrid between the latter plants, but on attempting to make the same cross

[1] C. Purdy (1916) advertises a very tall form of *H. Maximiliani*, said to grow to 11 ft. in height. Is this perhaps a hybrid?

artificially at Lafayette, he was unable to get any seed. *H. Dalyi*, according to FARWELL, is a variety of *H. Maximiliani*, related to it much as var. *oppositifolius* Farwell is related to *H. giganteus*. An effort should be made, however, to raise a *giganteus*×*Maximiliani* hybrid. *H. ambiguus* (Gray) Britton is supposed to be a hybrid *giganteus*× *divaricatus*, or at any rate to have *giganteus* as one parent. THELLUNG records a garden hybrid *laetiflorus*×*rigidus*, and the plant called *H. serotinus* Tausch (1828) is supposed to be *strumosus*×*rigidus*. Evidently there is a great deal to be done, both in the field and in the garden, before we can reach a fairly clear understanding of this subject. It seems possible that in this genus the origin, through hybridization, of distinct plants, with the attributes of species, may be demonstrated.—T. D. A. COCKERELL, *University of Colorado, Boulder, Colo.*

RELATIONSHIPS WITHIN THE RHODOSPOREAE

During the last 15 or 20 years the author has studied the structure of 30 to 40 species of *Pluteus* and 4 or 5 species of *Volvaria*. In all of these species, without a single exception, the trama of the lamellae presents a curious and interesting structure. In the majority of the Agaricaceae, the trama hyphae of the lamellae lie, in general, in a parallel direction, as in *Mycena*, *Tricholoma*, *Collybia*, *Inocybe*, *Entoloma*, *Leptonia*, etc. In *Russula* and *Lactarius* many of the cells are so swollen that the trama of the lamellae presents a vesiculose appearance. In *Amanita* the hyphae show a strong divergence from the median plane toward the subhymenium as they descend in the trama.

In *Pluteus* and *Volvaria*, on the other hand, the most prominent hyphae *converge* as they descend in the trama of the lamellae. Along the median plane of the lamella there can usually be seen, in section, a layer of hyphae (sometimes more slender) against which these prominent cells converge. Attention was called to this peculiar structure in *Pluteus seticeps* in 1902,[2] but no interpretation was offered as to its origin or significance.

During the summer of 1917, Professor LEVA B. WALKER, of the University of Nebraska, while studying the development of *Pluteus*

[2] See *Leptonia seticeps* Atkinson, Jour. Myc. 8:116. 1902. Further collections and studies of this species show that it is a *Pluteus*. While the gills are attached to the stem before the expansion of the plant, they become free, rounded behind, and distant from the stipe. The stipe also easily separates from the pileus, and other structural characters are clearly those of *Pluteus*. It is therefore *Pluteus seticeps* Atkinson, ined.

admirabilis Pk., in this laboratory, discovered the origin of this structure. These prominent hyphae are long, stout, subcylindrical cells, which arise from the inner cells of the subhymenium and extend downward, converging by their free ends against the trama, often compressing it into a thin layer. In some species the free ends of these cells are subcapitate, with a slight constriction below the capitulum. It is clear, therefore, that these peculiar structures in the trama of the lamellae of *Pluteus* and *Volvaria* are internal cystidia of a special kind. They are different in form from the other types of cystidia which project outward beyond the surface of the hymenium in many species. The origin and development of these internal cystidia will be described by Professor WALKER in a forthcoming paper on the development of *Pluteus admirabilis*.

The presence of these numerous internal cystidia, giving a distinct structural aspect to the trama of the lamellae in *Pluteus* and *Volvaria*, indicates that there is a very close phylogenetic relation between these two genera. In both genera the stipe is separable from the pileus, and the spores are of the same type, being smooth, globose, or slightly elongated. No true species of *Annularia* have been examined in the fresh condition to determine whether or not internal cystidia are present. It cannot now be stated with confidence to which group it belongs, but other morphological features indicate that it is more closely allied to *Pluteus* and *Volvaria*. In the other genera of the Rhodosporeae the stipe is not separable from the pileus, the spores are angular, and these peculiar internal cystidia are absent.

In the Rhodosporeae, therefore, there are at least two distinct phyletic lines. The relation of the genera to these two phyletic lines may be represented as follows.

I. PLUTEINAE.—Pileus easily separable from the stipe; lamellae with numerous internal cystidia converging as they descend and sometimes nearly obliterating the trama by pressure; spores smooth. *Pluteus* and *Volvaria* (?*Annularia*).

II. ENTOLOMATINAE.—Pileus not separable from the stipe; lamellae without numerous internal cystidia, trama normal; spores angular. *Entoloma*, *Leptonia*, *Clitopilus*, *Eccilia*, *Nolanea*, and *Claudopus*.—GEO. F. ATKINSON[3]

[3] This paper was received from the late Professor ATKINSON in January 1918.

CURRENT LITERATURE

MINOR NOTICES

British lichens.—The first edition of part first of this work appeared in 1894. The catalogue was done by JAMES M. CROMBIE, who accomplished an excellent piece of work, viewed from the standpoint of the lichenists of his day and the generation preceding him. Part second was to have appeared shortly from the pen of CROMBIE, but his death prevented. Later the task was continued by ANNIE LORRAIN SMITH, and the second part appeared in 1911, following in part the methods of the author of the first part, on which it was an improvement as a whole. In 1918 the revision of part first by Miss SMITH[1] appeared.

The volume contains an introduction dealing with the nature of the lichen and a catalogue of the British lichens (pp. 520). The work of the original author has been thoroughly revised, and the volume contains much new material and marked changes in classification. There are usable keys, and the descriptions have dropped most of the antiquated phraseology of the lichenist of the recent past. They will be more acceptable, therefore, to the botanist of today, who necessarily has been somewhat appalled at the technical language of the lichenist. The 71 plates represent a large amount of work and will be found helpful to the student. Unfortunately, the author's conception of the nature of the lichen is wrong and therefore unfortunate in a work of first rank. In America, at least, and we believe in Europe as well, there has been a marked recent trend of opinion among students of lower plants to the effect that the dual hypothesis regarding lichens is untenable, and that the lichen must be a fungus after all, parasitic on an alga. However, the introduction on the nature of the lichen is a very minor feature of the work, and the publication of this revision gives us from the pen of one person a complete, creditable, and very useful catalogue of the British lichens. Although the catalogue in two volumes deals primarily with the lichens of a limited area, the work will be found useful in the study of the lichens of America and other regions.—BRUCE FINK.

American gall insects.—FELT[2] has published an excellent and usable key to the American gall insects. It is arranged with reference to the host plants on which the galls occur and will be of great value to every botanist who has

[1] SMITH, ANNIE LORRAIN, A monograph of the British lichens. Vol. I. 8vo. pp. xxiv+520. *pls. 71. figs. 11.* The British Museum. 1918.

[2] FELT, E. P., Key to American gall insects. N.Y. State Museum Bull. 200. pp. 310. *pls. 16.* 1917.

occasion to collect and determine plants or to study abnormal plant growths. It is the only reasonably complete publication of the kind in America. There are a total of 1439 species, most of which can very readily be recognized by means of the key, the 250 text illustrations, and the 16 full page plates. The publication is indexed both with reference to the host and the parasite.—Mel. T. Cook.

North American Flora.—The sixth part of volume 22 contains the conclusion of Rosaceae by Rydberg, including genera 54 to 57, much the largest genus being *Rosa*, with 129 species, 23 of which are described as new. The part closes with 26 pages of additions and corrections to the volume.

The first part of volume 32 contains the beginning of Rubiales by Standley, 8 genera of the Rubiaceae being presented. Much the largest genus is *Rondeletia*, with 109 species, 8 of which are described as new. Among the remaining genera 8 new species are described.—J. M. C.

NOTES FOR STUDENTS

Secondary dormancy in seeds.—Kidd and West[3] have continued the study of the controlling action of carbon dioxide on the germination of seeds of *Brassica alba*. In two previous papers by the senior author it has been shown that low concentrations of carbon dioxide inhibit the germination of seeds, and that temperature and oxygen pressure determine the concentration necessary to inhibit germination. In normal oxygen pressure 2–4 per cent carbon dioxide will inhibit germination at 3° C., while at 20° C. it requires 20–25 per cent. At 17° C. it requires 9–12 per cent carbon dioxide to inhibit with 5 per cent oxygen pressure and 20–25 per cent carbon dioxide with 20 per cent oxygen pressure. All seeds studied, except *Brassica alba*, germinate normally as soon as the carbon dioxide is removed, while *B. alba* remains dormant after the carbon dioxide is removed. The authors term this "secondary dormancy," in agreement with the usage of this term by Crocker.

In the production of secondary dormancy the authors note the following general facts: (1) secondary dormancy is not produced if oxygen is absent during the primary period of inhibition or if carbon dioxide has been used in too high concentration; (2) conditions during the primary period of inhibition which prevent subsequent occurrence of dormancy are the ones that exercise injury on the radicle; (3) 100 per cent dormancy is obtained only within narrow limits of carbon dioxide and oxygen pressure. Secondary dormancy is not produced by a change in the permeability of the coats to gases or water, or to an increase in their breaking strength, but by a change in the embryo

[3] Kidd, F., and West, C., The controlling influence of carbon dioxide. The production of secondary dormancy in seeds of *Brassica alba* following treatment with carbon dioxide and the relation of this phenomenon to the question of stimuli in growth phenomena. Ann. Botany **31**:457–487. 1917.

by which it becomes less responsive to germinative conditions. The following conditions caused the secondarily dormant seeds to germinate: removal or partial removal of the testa; redrying of the soaked seeds; short exposures to high or low temperatures; treatment with acids (especially n 0.01 HCl and propionic); treatment with high concentrations of carbon dioxide followed by germination in air. High partial pressures of oxygen had no effect on the germination of secondarily dormant seeds.

The authors give the following interpretation of this work: "It will be seen that the main interest of this communication centers around the causes underlying the initiation of growth rather than in the conditions of dormancy. In considering this question of growth in the case of seeds of *B. alba*, our experiments show clearly that there is no question of limiting factors. We have been able to trace no limiting factor responsible for the non-germination of white mustard seeds showing secondary dormancy. We find ourselves rather in the presence of facts which emphasize a conception of stimulus. It has been seen that widely different treatments, quite unclassifiable in any feature other than that they all result in injury and death, if carried too far, excite germination and growth of white mustard seed. It appears to us probable that some return will have to be made to this conception of stimulus in plant physiology generally, and that in any experimental analysis of the living plant, as a unit and in relation to its life-cycle, the idea of limiting factors, which has so long dominated the minds of plant physiologists, will have to be modified."—Wm. Crocker.

Chondriosomes in plants.—Investigations dealing with chondriosomes have become so numerous that it seems worth while to make a brief summary of the results obtained. As might be expected, a few structures of different nature have been called by the same name; but a host of names have been applied to the same structure, so that we have mitochondria, chondriosomes, chondriomites, chondriokonts, chromidia, sphaeroblasts, histomeres, plasmosomes, cytomicrosomes, etc. The "chondr," meaning a small grain, was chosen because most of the bodies are in the form of small granules; the "mito," meaning thread, is often suggestive, because the granules have a tendency to become arranged in rows. The terms mitochondria and chondriosomes will probably survive, and if a choice should be made between these two, it should be the latter, since it is noncommittal; while the fact that the threadlike arrangement is by no means universal is an objection to the term mitochondria. The name chromidia was applied because the writer believed that the granules were portions of the chromatin extruded from the nucleus. Such granules certainly occur in animals and possibly in plants, but they are not the same structures as the chondriosomes.

A historical résumé of the subject, with a very complete bibliography up to 1914, was compiled by Cavers,[4] and in an investigation upon the rela-

[4] Cavers, F., Chondriosomes (mitochondria) and their significance. New Phytol. 31:170–180. 1914.

tion between chondriosomes and plastids, MOTTIER[5] has brought the literature up to 1918.

LA VALETTE ST. GEORGE, working upon the male cells of insects, gave the first description of chondriosomes. He introduced the term cytomicrosomes. MEVES, in 1904, gave the first description for plants, using the tapetal cells of anthers of *Nymphaea* for material. LEWITSKI, in 1910, was first to claim that chondriosomes give rise to plastids. A little later he made a comparative study upon living and fixed material, showing conclusively that the bodies are present in living cells. The investigation by MOTTIER, to which reference has already been made, proves that some chondriosomes give rise to chloroplasts and leucoplasts. He also believes that the chondriosomes are permanent organs of the cell, of equal rank with the nucleus. Of course he recognizes that chloroplasts and leucoplasts also multiply by division. His claim that chondriosomes are concerned in the transmission of hereditary characters does not seem to be so well supported. Some investigators have suggested that chondriosomes transmit characters of the cytoplasm and that the chromosomes transmit characters of the nucleus.

It seems to be established that chondriosomes are not artifacts, that they multiply by division, and that some of them give rise to plastids. Their rôle in heredity, if they have any, still remains to be demonstrated.—C. J. CHAMBERLAIN.

Trimorphism of Pontederia.—The family Pontederiaceae is notable as containing the only known heterostyled species among monocotyledons (with possibly one exception), and is further remarkable among heterostyled plants as furnishing the only recorded examples of distinctly zygomorphic flowers in such plants. HAZEN[6] has recently published interesting observations on *Pontederia cordata* L. LEGGETT had reported in 1875 that this species was trimorphic, and the present paper is a detailed study of the flower forms, pollination, insect visitors, etc.

The tubular perianth is slightly zygomorphic and in all 3 flower forms presents 2 sets of stamens: a longer set of 3 on the anterior side of the flower, and 3 short-stalked stamens on the posterior side of the flower. In 2 of the flowers the upper stamens protrude beyond the open perianth. The long-styled stigma reaches a height of 12–13.5 mm., the mid-styled form 7–8 mm., and the short-styled form 3–3.5 mm. above the base of the ovary. The ratios of the average heights of the 3 lengths of pistils are approximately as 100, 60, and 22.

While the arrangement of parts is different in each of the 3 flower forms, it results in 2 sets of stamens adjusted to each length of pistil. The 6 legitimate crosses which may take place between the 6 sets of stamens and the 3 different

[5] MOTTIER, D. M., Chondriosomes and the primordia of chloroplasts and leucoplasts. Ann. Botany 32:191–214. *pl. 1.* 1918.

[6] HAZEN, TRACY E., The trimorphism and insect visitors of *Pontederia*. Mem. Torr. Bot. Club 17:459–484. 1918.

pistils are such that each flower type may be pollinated by either of the other 2 flower forms. Moreover, the flowers are placed on the axis of the spike so nearly horizontal as to lessen the probability of self-pollination.

The microspores are ellipsoidal in form and the different sets of stamens show marked differences in size of pollen grains, the higher anthers having the larger pollen, the middle ones intermediate, and the short-stalked stamens the smallest spores. This relation suggests a correspondence with the 3 types of stigmas. Averaging a large number of spores it was found that the mean diameters of the 3 sizes of pollen grains were as 100, 80, and 51, and their volumes respectively as 100, 53, and 14. Recalling HALSTED's work on *Eichhornia crassipes*, in which he found that all sizes of pollen grains germinated if given sufficient time, but that the larger spores germinated much more promptly than the smaller, HAZEN suggests that prompt germination would be of great advantage in the long-styled *Pontederia* flowers in which the flowers wither so quickly that a slow germinating spore might not have time to function.

The author lists observed insect visitors, naming 10 Lepidoptera and 4 Hymenoptera, the least skipper, *Ancyloxypha numitor* Fabr., being the most frequent visitor. Experimental work by the author is in progress on the relative fertility of the different flowers with various pollen combinations, and its publication is awaited with interest.—R. B. WYLIE.

Phototropism.—Miss PARR,[7] working in HOTTES' laboratory of the University of Illinois, has done an excellent piece of quantitative work on the response of *Pilobolus* to light. The literature on phototropism has been full of conflicting statements and theories, very largely due to the lack of quantitative work of the type done by Miss PARR. This work does much to show the reasons for these diverse views and to lay the foundations for substantial progress. The physics department of the University assisted in the control of the delicate instruments used in the measurements of light. It is very desirable at this stage of plant physiology that we get the more general cooperation of well-trained physicists and chemists to aid in transforming plant physiology from a qualitative to a quantitative science. The results of the work can best be presented by quoting the summary: (1) *Pilobolus* responds to the light of all regions of the visible spectrum; (2) the presentation time decreases gradually from red to violet, and there is no indication of intermediate maxima and minima; (3) the presentation time does not vary in direct ratio with the measured value of the energy of the light in the different regions of the spectrum; (4) the presentation time varies in inverse ratio to the square roots of the wave-frequency; (5) the product of the square root of the frequency times the presentation time decreases with the decrease in the energy value of the spectral regions and is an approximate constant for a given light source; (6) the spectral energy in its relation to presentation time may be expressed approximately in

[7] PARR, ROSALIE, Response of *Pilobolus* to light. Ann. Botany 32:177–205. 1918.

the Weber-Fechner formula, if the wave-frequencies be made a function of the constant; (7) the relation of the spectral energy to the presentation time may also be approximately expressed in the Tröndle formula, the wave-frequency being made a function of the constant.—Wm. Crocker.

Breeding for disease resistance.—It has been a popular impression that newly produced disease resistant varieties will gradually lose their immunity in later generations. The idea was that such new varieties might sometimes become slightly infected; this short sojourn of the disease organism in the normally immune host would enable the former to adapt itself to the new conditions and gradually acquire virulence, until finally a new biologic form was developed to which the host in question was quite susceptible. Evans[8] carried the same idea further when he found that a cross between resistant and susceptible races of wheat produced a hybrid even more susceptible to rust than the susceptible parent. Furthermore, rust from the hybrid could now infect the immune parent. Such facts were very discouraging, since they indicated that the artificial breeding of resistant crop plants is rapidly overtaken by the natural breeding of new biologic forms of the disease organism.

Particularly acceptable, therefore, is the work of Stakman, Parker, and Piemeisel,[9] who find that wheats resistant to rust remain resistant regardless of the previous history of the rust; the gap between immune and susceptible varieties is not bridged by transitional varieties or by artificial hybrids. "Resistance is rather an hereditary character, which cannot be produced by the accumulation of fluctuating variations within a susceptible line, nor broken down by changes in the host or parasite." Acceptable as such a conclusion may be, both to commercial breeders and to academic geneticists, it is very questionable how widely it may be applied. It will be difficult, although not hopeless, to explain away much of the contrary evidence.—Merle C. Coulter.

Nature of monocotyledonous leaves.—Mrs. Arber[10] has presented the results of an anatomical investigation of the phyllode theory of the monocotyledonous leaf. According to DeCandolle, it is equivalent to the leaf-base and petiole of a dicotyledonous leaf, but Mrs. Arber believes that certain monocotyledonous leaves are still further reduced in that they are equivalent to leaf-bases only. In case the monocotyledonous leaf shows a distinction of petiole and blade, Henslow suggested that the blade is merely an expansion

[8] Evans, I. B. P., South African cereal rusts, with observations on the problem of breeding rust resistant wheats. Jour. Agric. Sci. 4:95–104. 1911.

[9] Stakman, E. C., Parker, John H., and Piemeisel, F. J., Can biologic forms of stem rust on wheat change rapidly enough to interfere with breeding for rust resistance? Jour. Agric. Research 14:111–123. *pls. 13–17.* 1918.

[10] Arber, Agnes, The phyllode theory of the monocotyledonous leaf, with special reference to anatomical evidence. Ann. Botany 32:465–501. *figs. 32.* 1918.

of the apical region of the phyllode and not homologous with the blade of a dicotyledonous leaf. Such a blade among monocotyledons Mrs. ARBER calls a "pseudo-lamina." Such theories have been devised to explain the parallel venation of monocotyledonous leaves. Attention is also called to GRAY'S suggestion that some gymnosperm leaves may be equivalent to petioles, and the further suggestion made that this may be applied specially to the Gnetales.

These views were tested by Mrs. ARBER in anatomical investigations, comparing scale-leaves, petioles, and phyllodes of dicotyledons with the leaves of monocotyledons, the conclusion being reached that the occurrence of inverted vascular bundles toward the adaxial face of a leaf may be an indication of "phyllodic morphology." Other indications of phyllodic anatomy are developed, and its systematic distribution shows that it does not occur with any frequency outside the Helobiae, Liliiflorae, and Farinosae. This distribution is taken to confirm the view that phyllodic anatomy is an ancient character, revealing the origin of the monocotyledonous leaf.—J. M. C.

Stomata.—REHFOUS[11] has published a detailed study of the stomata of many groups. The details are too numerous for citation, but some of the general conclusions may be indicated. He is convinced that stomata are of first importance in indicating phylogeny and relationships. Their structure he claims is very constant within a group, numerous examples of this being given. For example, the structure of the stomata of the Amentiferae shows that they are nearer the level of the dicotyledons than of the gymnosperms or pteridophytes. In the same way it is shown that the Polypodiaceae constitute a special group, and that the Osmundaceae, Gleicheniaceae, and Schizeaceae approach more nearly the higher plants. A close resemblance is found between the stomata of cycads and conifers, leading to the conclusion that these groups are of common origin. Numerous illustrations of claimed relationships within great groups are either confirmed or contradicted. Several new types of stomata are described, among which those of *Polypodium*, *Platycerium*, *Cycas*, and *Casuarina* may be cited. In connection with the last named genus it is pointed out that its stomata are related to those of certain monocytoledons, as the grasses and certain of the xerophytic Liliaceae. The contribution is a valuable assemblage of facts in reference to the structure of stomata, accompanied by clear illustrations. The conclusions drawn from these facts are open to discussion.—J. M. C.

Water conduction in trees and shrubs.—FARMER[12] has published the results of an investigation of the comparative efficiency of the wood as a water-conducting tissue in about 60 species of plants, chiefly trees and shrubs. The

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[11] REHFOUS, LAURENT, Étude sur les stomates. Univ. Genève, Inst. Bot. IX. no. 6. pp. 110. *figs. 125.* 1917.

[12] FARMER, J. BRETLAND, On the quantitative differences in the water-conductivity of the wood in trees and shrubs. Proc. Roy. Soc. B. **90**:218–250. 1918.

intake of water by the roots and its transpiration from the leaves have been much investigated, but "the behavior of the wood as the intervening conducting channel has almost entirely been neglected." The method used was to measure the amount of water passing in a given time and at standard pressure through a definite length of twig, the area of the cross-section of the wood being carefully measured. The paper includes two parts, one dealing with evergreens and the other with deciduous plants.

Some of the results are as follows. The specific conductivity of evergreens is relatively low, while that of deciduous plants is relatively high, and with a higher fluctuation. Some of the deciduous trees are more influenced by environmental conditions than are others. Considerable difference, in a lowering of conductivity, was found between the adult wood of the tree and that of "leaders" of young trees, a difference which becomes "exaggerated" in the main shoot of most climbers. The wood of arborescent monocotyledons was found to be defective in water-conductivity. The facts suggest that the lower conductivity of evergreens may be attributed to their narrow and short vessels.—J. M. C.

The Journal of General Physiology.—Many will welcome a new journal of general physiology.[13] Both plant and animal physiology have suffered from being too little related and treated as distinct subjects. Such a publication will aid in bringing them into closer relation. This journal is sure of sufficient financial support and no doubt able editorship. Its aim is stated as follows: "*The Journal of General Physiology* is devoted to the explanation of life phenomena on the basis of the physical and chemical constitution of living matter." The first number contains the following articles: On the dynamics of photosynthesis, W. J. V. Osterhout and A. R. C. Haas; A method of studying respiration, W. J. V. Osterhout; The antagonism between thyroid and parethyroid glands, E. Uhlenhuth; Difference in the action of radium on green plants in the presence and absence of light, C. Packard; Amphoteric colloids, J. Loeb; A theory of the mechanism of disinfection, hemolysis, and similar processes, S. C. Brooks; The law controlling the quantity of regeneration of the stem of *Bryophyllum calycinum*, J. Loeb; Reversal of reaction by means of strychnine in planarians and starfish, H. R. Moore; Light and the muscle tonus of insects; the heliotropic mechanism, W. E. Garrey; Lutear cells and hen-feathering, Alice M. Boring and T. H. Morgan.—Wm. Crocker.

Embryo sac and fertilization in Oenothera.—Ishikawa[14] has investigated the behavior of the gametophytes and the fertilization phenomena in *O. nutans*

[13] The Journal of General Physiology, editors, Jacques Loeb and W. J. V. Osterhout. Published bimonthly by the Rockefeller Institute for Medical Research. Vol. I. No. 1. September 1918. Subscription $5.00.

[14] Ishikawa, M., Studies on the embryo sac and fertilization in *Oenothera*. Ann. Botany 32:279–317. *pl.* 7. *figs.* 14. 1918.

and *O. pycnocarpa* and their hybrids, both of which species were formerly included in *O. biennis*. Many valuable confirmatory details need not be cited, but the following may be mentioned. The embryo sac is 4-nucleate, lacking antipodals and one of the polar nuclei, and this condition was found not only in *Oenothera*, but also in *Ludwigia*, *Gaura*, *Godetia*, and *Circaea*. The author regards it as a diagnostic character of Onagraceae, and therefore would exclude *Trapa*, with its normal 8-nucleate sac, from the family. This condition in Onagraceae he thinks may have been produced by mutation, but not by adaptation. The pollen tube enters the synergid and the "mixed plasma" flows out and spreads over the egg. The cytoplasm of the pollen grain was found to contain an immense number of minute starch grains, which migrate through the pollen tube, enter the synergid, and finally disappear. The male nucleus is inclosed in a distinct plasma sheath until it reaches the egg. The synergid and the upper two-thirds of the egg have a distinct cellulose membrane, the lower part of the egg acquiring it after fertilization. Self-sterility of some hybrids is said to be due to the feeble growth of the pollen tube.—J. M. C.

Histology of phloem.—There has been a tendency in recent years to assume that the doctrine of recapitulation is a law as valid and invariable as the laws of physics and chemistry, and to use it as a reliable short cut in the study of the evolution of plants. However, it is to be emphasized that a law is a statement of fact, not a theory or working hypothesis. If the doctrine of recapitulation and similar generalizations are to be accepted as true laws they must be capable of statistical or experimental proof. MacDaniels[15] points out that, although in a considerable number of woody dicotyls which he studied there is no fundamental difference between the type of sieve tube found in seedlings and first annual rings and that found in the mature condition, the remaining forms possess a presumably less primitive type of structure in the earlier than the later stages of ontogeny. Furthermore, he shows that there is no close parallelism in the specialization of sieve tubes, vessels, and floral structures. It has been a common morphological fallacy to assume that because the evolution of a selected structure progresses apparently in a given direction the sums of all structures (organisms) are moving in a similar direction. MacDaniels' comprehensive and painstaking piece of work is a valuable contribution to our knowledge of the histology of phloem.—I. W. Bailey.

Enzyme secretion.—The influence of such inorganic salts as the nitrates, chloride sulphates, and monobasic phosphates of sodium and potassium, and the chlorides and sulphates of calcium and magnesium on the secretion of diastase by *Penicillium camembertii* has been investigated by Robbins.[16]

[15] MacDaniels, L. H., The histology of the phloem in certain woody angiosperms. Am. Jour. Bot. 5:347–378. 1918.

[16] Robbins, W. J., Influence of certain salts and nutrient solutions on the secretion of diastase by *Penicillium camembertii*. Amer. Jour. Bot. 3:234–260. 1916.

The general results show decrease in the amount of digestion of starch by the fungus in the presence of low concentrations (M/10,000 and M/100,000) of the chlorides and sulphates. The view is taken that the decreased digestion is caused by decreased secretion of diastase rather than by inhibition of the activity of secreted diastase. Potassium salts decrease secretion more than corresponding sodium salts. Experiments with nutrient solutions instead of single salts showed the same general effect, decreased secretion. No evidence was found to support the idea that calcium or potassium is intimately related to diastase formation. On the other hand, nitrogen may possibly have some relation to enzyme formation. Nitrates added singly increase the actual amount of starch digestion, but since the mycelial growth is much increased, there is really less digestion per unit of dry weight of mycelium.—C. A. SHULL.

Reaction of the medium and nitrogen assimilating organisms.—FRED and DAVENPORT[17] have studied the relation of the legume bacteria and *Azobacter* to low concentrations of acids and alkalies. When sulphuric acid was added to the nutrient solutions, the following hydrogen ion concentrations were found to be critical for the various legume organisms: alfalfa and sweet clover, P_H 4.9; garden pea, field pea, and vetch, P_H 4.7; red clover and common beans, P_H 4.2; soy beans and velvet beans, P_H 3.3; lupines, P_H 3.15. The authors believe a correlation exists between the acid resistance of the bacteria and the acid resistance of the higher plant with which they are associated. These organisms are not injured by normal alkali additions to the culture medium until the addition is about 10 times that of sulphuric acid producing injury. There seems to be little difference in the several strains as to the alkali resistance.

Azobacter is limited to a much narrower range of reaction than are the legume organisms, the critical limits being 6.5 P_H for acid and 8.6 P_H for alkali. It is to be regretted that the reaction was not determined by the gas chain as well as by the colorimetric method.—WM. CROCKER.

Transpiration.—DUGGAR and BONNS[18] have issued a third paper from the Missouri Botanical Garden on the effect of a film of Bordeaux mixture and other films on the transpiration of leaves. In potted mesophytes such a film increases generally the transpiration at night, but has less or no effect during the day. Similar behavior is shown by excised leaves. In *Cyperus esculentus*, a plant of xerophytic surface modification, such films have no effect on transpiration rate. The writers offer as tentative the following explanation: the film of Bordeaux mixture on the surface of a plant in a state of guttation acts more or less as a bibulous surface, taking water directly from the interior of the plant, through at least some continuous water channels

[17] FRED, E. B., and DAVENPORT, AUDREY, Influence of reaction on nitrogen-assimilating bacteria. Jour. Agric. Research 14:317–336. 1918.

[18] DUGGAR, B. M., and BONNS, W. W., The effect of Bordeaux mixture on the rate of transpiration. Ann. Mo. Bot. Gard. 5:153–176. 1918.

established by means of the open water-suffused stomata. This would account for the effectiveness of the film at night and for its lack of effectiveness with *Cyperus* with its very narrow stomata. The authors state that there are difficulties in the incipient guttation explanation as applied to excised leaves.—Wm. Crocker.

Turgor movements.—Blackman and Paine,[19] by use of a special conductivity cell, have studied the conductivity of the liquid extruded from the lower half of the excised pulvinus of *Mimosa pudica* due to the shock stimulus. The shock response gives an increase in conductivity, but not nearly enough to attribute the contraction to increased extrusion of solutes. They believe, therefore, that the contraction is due to a sudden condensation of solutes within the pulvinal cells of the lower half of the pulvinus. They consider the conductivity method far superior to the plasmolytic method used by previous authors, for it answers directly the amount of movement of solutes. Under certain conditions they get autonomic movements of this organ similar to those of the leaflets of *Desmodium gyrans*. A slow rise of temperature up to 50° C. shows little increase in exosmose of electrolytes from this organ. The increase of permeability at higher temperatures seems to be due to lethal irreversible changes.—Wm. Crocker.

Alternation of generations in Padina.—*Padina variegata*, one of the Dictyotaceae, is abundant at Beaufort, North Carolina, where it has been studied by Wolfe.[20] Sperms, eggs, and tetraspores are borne on 3 separate plants which look alike in the vegetative condition, but which are easily recognized during reproduction. Tetraspores give rise to only male and female plants in approximately equal numbers, so that sex is probably predetermined during the reduction division in the tetraspore mother cell. Fertilized eggs produce only tetrasporic plants, so that there is an alternation of sporophyte and gametophyte generations. Eggs often germinate without fertilization, but plants of such parthenogenetic origin do not mature. It would be interesting to know the chromosome numbers, especially in the parthenogenetic plants, and we hope that Wolfe, who is familiar with the cytological technique of the algae, will investigate this phase of the problem.—C. J. Chamberlain.

The luminous moss.—Toda[21] has made a physiological study of *Schistostega osmundacea*, the so-called luminous moss, his material having been obtained from a cave in Japan. He found the optimum intensity of light as well as the minimum and maximum intensities in terms of Bunsen's unit. In a dark place

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[19] Blackman, V. H., and Paine, S. G., Studies in the permeability of the pulvinus of *Mimosa pudica*. Ann. Botany 32:69–85. 1918.

[20] Wolfe, J. J., Alternation and parthenogenesis in *Padina*. Jour. Elisha Mitchell Scientific Soc. 34:78–109. 1918.

[21] Toda, Viscount Yasumochi, Physiological studies on *Schistostega osmundacea* (Dicks) Mohr. Jour. Coll. Sci. Tokyo 40:no. 5. pp. 30. *pls. 2*. 1918.

the protonema can live for 7 months without producing a leafy shoot. He observed also the movement of "chomatophores," which became scattered in a day when the protonema is placed in light, and when the direction of light is changed they all turn toward it in 7–10 days. Blue and violet light proved to be more favorable than any other of the visible rays, excepting of course white light. The optimum temperature for the development of the leafy shoot is 16–25° C.; the protonema does not die so long as the temperature is above −20.5° C., but the leafy shoot dies at −18° C. The spore at a temperature of 16–25° C. germinates in one month.—J. M. C.

Angiosperm wood lacking vessels.—BAILEY and THOMPSON,[22] in continuing their work on certain genera of angiosperms in which true vessels are absent from the normal wood of the stem, have obtained additional evidence. Their attention had been called to the occurrence of vessel-like structures in injured roots of a species of *Drimys*, which might indicate that the ancestors of the 3 genera investigated possessed true vessels. An examination of these structures has led to the conclusion that they are not vessel-like in structure, but are typical tracheids, which occur as well in uninjured stems of the 3 genera. They maintain, therefore, that true vessels do not occur in the xylem of these genera, and that there is no evidence that their ancestors possessed true vessels.—J. M. C.

Permeability.—PAINE and SAUNDERS[23] find that the testa of the pea is impervious to various reagents dissolved in water (copper ferrocyanid, sodium chloride, safranin) due to a waxy bloom deposited on the outer surface. This bloom is easily rubbed off so that the testa becomes pervious. In the wrinkled peas the bloom rubs off on the wrinkles, leaving the depressions still impervious, while in the smooth pea the bloom rubs off uniformly on the whole surface. It is interesting to find such a superficial layer responsible for the peculiar permeability characters of seed coats, for these characters are generally determined by deeper layers.—WM. CROCKER.

Agaricaceae of Michigan.—KAUFFMAN,[24] in connection with his very full presentation of the Agaricaceae of Michigan, has monographed *Russula* (pp. 118–167), *Pholiota* (pp. 289–314), and *Cortinarius* (pp. 314–442), as represented in the state. In *Russula* he recognizes 53 species, 3 being new and 27 edible; in *Pholiota* 26 species, 4 of which are edible; in *Cortinarius* 154 species, 13 of which are new and 10 edible. As an illustration of the activity of CHARLES

[22] BAILEY, I. W., and THOMPSON, W. P., Additional notes upon the angiosperms *Tetracentron*, *Trochodendron*, and *Drimys*, in which vessels are absent from the wood. Ann. Botany 32:503–512. *pl. 16. figs. 9.* 1918.

[23] PAINE, S. G., and SAUNDERS, L. M., On a peculiarity exhibited by the testa of wrinkled peas. Ann. Botany 32:175. 1918.

[24] KAUFFMAN, C. H., The Agaricaceae of Michigan. Mich. Geol. and Biol. Survey, Publ. 26. Biol. Series 5. December 1918.

PECK in these groups it is interesting to note that he is credited with 16 species in *Russula*, 11 in *Pholiota*, and 62 in *Cortinarius*, and this has to do only with Michigan species.—J. M. C.

Seedling anatomy.—HOLDEN and BEXON[25] have begun a series of studies on the anatomy of teratological seedlings. The first paper deals with seedlings of *Cheiranthus Cheiri*, which showed "cotyledonary abnormality ranging from hemitricotyly to tetracotyly." The conclusion was reached that there are at least two methods of cotyledonary increase, cotyledonary fission and dichotomy of the growing point of the cotyledon. A third method is somewhat doubtfully suggested, namely "the downward displacement of one or more epicotyledonary leaves."—J. M. C.

Apogamy in Camptosorus.—Mrs. BROWN[26] has described a case of apogamy in *C. rhizophyllus* that occurred in cultures to determine if apogamy could be induced by the modification of external conditions. The apogamous outgrowth was in general a cylindrical process, with some interesting details as to shape and structure, in which a cluster of tracheids appeared. Previous experimental work had indicated that bright light and relative dryness were the factors involved; but in this case low nutrition seemed to be more important than either.—J. M. C.

Tropical species of Eupatorium.—ROBINSON[27] has published the results of a study of *Eupatorium* as displayed in the American tropics. The wealth of species illustrates how much of the flora of the world remains to be discovered. There are 39 new species described, in addition to new varieties. He has included also a revision of the Colombian species, recognizing 93 species distributed among 7 sections. "Keyed recensions" are given also of the species of Venezuela (35) and of Ecuador (50).—J. M. C.

The orchids of Java.—SMITH,[28] in a fifth paper on the orchids of Java, continues to bring to light the remarkably rich orchid flora of that island. He discusses 61 species representing 27 genera, including 38 new species and 2 new genera (*Chroniochilus* and *Saccolabiopsis*).—J. M. C.

A new genus of Compositae.—PRITZEL[29] has published a new genus (*Basedowia*) of Compositae from Australia. It resembles *Helichrysum*, as the name (*B. helichrysoides*) suggests. The genus is named for HERBERT BASEDOW, state geologist of South Australia.—J. M. C.

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THE BOTANICAL GAZETTE

Editor: JOHN M. COULTER

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JOHN M. COULTER

With the assistance of other members of the botanical staff of the University of Chicago

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THE
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APRIL 1919

AFTER-RIPENING AND GERMINATION OF SEEDS OF TILIA, SAMBUCUS, AND RUBUS

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R. C. ROSE

Introduction

This paper gives the results of an attempt to determine the conditions favoring the after-ripening and germination of the seeds of *Tilia americana*, *Sambucus canadensis*, and *Rubus Idaeus*, and some of the chemical processes involved therein. Since layering of these seeds usually results in very low percentages of germination, it was thought possible to discover some other means of overcoming their dormancy.

Literature

The present state of our knowledge of the causes of delay in germination, and the means of overcoming it, is admirably summarized in a recent paper by CROCKER (5). He divides seeds which show delay in germination into 7 classes. In 3 of these the seed coats play the important rôle, while in the fourth dormancy is occasioned by the embryo. Where dormancy or poor germination is due to the seed coat, the use of concentrated sulphuric acid as a carbonizing agent has become a common practice. ROSE (23) mentions ROSTRUP (24) as the first to resort to this treatment, and lists TODARO (25), HILTNER (12), JARZYMOWSKI (14), BOLLEY (2), and LOVE and LEIGHTY (19) as investigators applying the same method. EWART (9) found this treatment effective with several

species of *Acacia*, as did CROCKER (unpublished work) with *Scirpus*. The length of time required by this treatment varies from a few minues to several hours, depending upon the resistance of the coats.

Boiling water or warm water, used as a forcing agent, has proved effective in a number of cases where hard-coatedness is the cause of the delay. BRUYNING (3), working with the seeds of *Ulex europaeus*, found that a treatment of 1–5 seconds with boiling water raised the percentage of germination from 13 for untreated seeds to 53·5–75·5 for treated seeds. HONING (13) obtained his best results with *Albizzia* seeds by soaking them in water at 60° C. for at least 3 hours, while with *Mimosa* 60–70° C. proved most effective, as did 70–75° C. for *Pithecolobium*. Soaking seeds of *Crotalaria* in warm water proved disadvantageous. BOLLEY (2) states that improvement in germination was obtained by this method if the exposure was not long enough to kill the embryo.

NOBBE (21) mentions ALEXANDER VON HUMBOLDT as the first investigator to use chemicals as forcing agents. From the time of HUMBOLDT (1793) up to 1873, the date of publication of NOBBE's book, many investigators used as forcing agents a great variety of substances, both organic and inorganic. The range of substances used is more interesting than the results obtained. Moreover, quickly germinating seeds were used and in such cases the effect of forcing agents is not so striking as where dormancy is involved. Of the more recent workers in this field, LEHMANN (16) was the first to emphasize the importance of chemical substances in connection with germination. He showed that the seeds of *Ranunculus sceleratus* are forced into germination by Knop's solution, by soil, by soil wet with weak solutions of hydrochloric acid, potassium hydroxide, ferric chloride, and hydrogen peroxide. Two years later GASSNER (10) found Knop's solution effective on unthreshed seeds of *Chloris ciliata*, and more recently (11) has shown that for several other seeds various nitrogen compounds, especially nitrites and nitrates, are effective forcing agents. *Chloris ciliata* was found to have a membrane impermeable to potassium nitrate and magnesium nitrate, and from this GASSNER concludes that the effect is upon the seed coat alone. LEHMANN (17) and LEHMANN and OTTENWÄLDER (18), working with seeds representing

a number of different families, showed that acids in low concentrations, especially hydrochloric acid, are effective forcing agents. CROCKER and DAVIS (6) obtained similar results for seeds of *Amaranthus* (unpublished work) and *Alisma*. Bases are equally effective for *Sagittaria* and *Alisma*, but not for *Amaranthus*. According to OTTENWÄLDER (22) bases exert an inhibitory effect on seeds of *Epilobium hirsutum*.

In those cases where a state of dormancy exists in the embryo itself (*Crataegus* and *Malus*), temperatures slightly above freezing have been found effective in hastening after-ripening (7). In *Crataegus*, as ECKERSON (8) has shown, the hypocotyl becomes more acid as after-ripening progresses; hence dilute acids hasten after-ripening by acting upon the hypocotyl directly.

Material

The seeds used in these experiments were gathered in the summer or the fall of 1916 and 1917. Each year those of *Sambucus* were all collected on the same day from neighboring plants. *Tilia* seeds of the 1916 crop were collected during October from trees growing on the dunes at the southern end of Lake Michigan. The 1917 crop was gathered during September from trees in the parks of Washington, D.C. The seeds of *Rubus* were collected during late June 1916 from neighboring plants of several varieties, but no attempt was made to keep those of the different varieties separate. Among the seeds of all 3 species were found many without embryos or with defective embryos. In most cases this fact accounts for the varying number of seeds used in the cultures. Approximately 60 per cent of *Rubus*, 75 per cent of the 1916 *Sambucus*, and 80 per cent of the 1916 crop of *Tilia* were viable. Not more than 5 per cent of the 1917 crop of *Tilia* and *Sambucus* were defective.

Histology and microchemistry of seed coats

SAMBUCUS: *Endocarp.*—The seed in cross-section shows in the lignified endocarp 3 regions: (1) the outermost, consisting of 3 or 4 layers of cells of irregular size and shape, with thin walls and large lumina; (2) a middle one of 1 or 2 layers of fibers in cross-section; and (3) an inner one of 1 or 2 layers of fibers in longitudinal section. *Seed coat.*—This consists of several layers of collapsed cells with

lignified walls; the cells contain a considerable quantity of reducing sugar.

TILIA: *Pericarp.*—This is composed of two layers: (1) a surface region of loose fibers with cellulose walls, and (2) a thicker region of lignified fibers. *Seed coat.*—This consists of 3 regions: (1) cells with suberized or cutinized walls; (2) one layer of palisade cells with (a) outer end walls of cellulose, (b) a lignified light zone, (c) a pectinized region, and (d) a lignified region; and (3) 3 or 4 layers of cells with walls which stain with ruthenium red and give the ceric acid test.

RUBUS: *Endocarp.*—This consists of 2 layers: (1) an outer layer, variable in thickness, of lignified fibers longitudinally arranged in cross-section of the fruit; (2) an inner region of 4 or 5 layers of lignified fibers transversely arranged in cross-section of the fruit. *Testa.*—This consists of 4 regions: (1) 1 layer of cushion-shaped cells with lignified walls; (2) 4 layers of collapsed cells with cellulose walls; (3) 1 layer of collapsed cells with thick pectinized walls; and (4) 1 layer of cells with cellulose walls which appear as a thickened outer wall of the endosperm.

Microchemistry

In table I are given the results of the microchemical tests made upon the endosperm and embryo of each of the kinds of seeds used. Owing to the lack of a sufficient number of germinating seeds of *Sambucus* several of the tests have not been completed. The storage materials in all the seeds are very similar, starch, fats, and protein being found in every case. In addition to these *Sambucus* contains amylodextrin. *Tilia* contains much more fat and phytosterol than either *Sambucus* or *Rubus*. The phytosterol shows up as a bright red layer around the fat globules when sections of the seeds are placed in concentrated sulphuric acid. Oxidase is present in the dry seeds in very small quantities, and in the germinating seeds benzidine gives a positive test only after several hours. Peroxidase, while present in dry *Tilia* seeds, is much more abundant in the germinating seeds. Dry seeds of *Sambucus* and *Rubus* give no peroxidase reaction. Catalase is found in both dry and germinating seeds of all 3 species.

TABLE I

Substance	Tilia				Sambucus				Rubus			
	Ungerminated		Germinated		Ungerminated		Germinated		Ungerminated		Germinated	
	Endosperm	Embryo	Endosperm	Embryo	Endosperm	Embryo	Endosperm	Embryo	Endosperm	Embryo	Endosperm	Embryo
Starch	+	++	++	+++	−	++	−	++	−	−	+	++
Amylodextrin	−	−	−	−	++	−	+	−	−	−	−	−
Reducing sugar	−	−	+	++	−	−	?	?	−	−	+	+
Protein reaction:												
Xanthoprotein	+	+	+	++	++	++	?	?	+	+	+	+
Biuret	++	++	++	++	+	+	?	?	++	++	+	+
Fat	+++	++	++	+	+	+	+	+	+	+	+	+
Phytosterol	+++	++	++	+	+	+	?	?	++	++	+	+
Tannin	−	−	−	−	−	−	−	−	++	+	++	+
Oxidase	−	−	+	++	−	−	?	?	−	−	+	+
Peroxidase	+	+	++	++	−	−	+	++	−	−	+	++
Catalase	+	+	+	+	+	+	+	+	+	+	+	+
Reaction	Neutral or acid	Neutral or acid	Alkaline	Acid	Alkaline	Alkaline	Alkaline	Acid	Acid	Acid	Acid	Acid

Conclusive determinations in regard to the reaction of fresh dry seeds of *Tilia* have not been made, but preliminary tests, where neutral red was used as an indicator, indicate that the endosperm and cotyledons are acid and the hypocotyl alkaline. Seeds kept in dry warm storage for 9 months show an acid reaction throughout. Hydrogen ion determinations, the data for which are given later, showed an acid reaction for the stored seed as well as for the germinating ones. As germination begins, the reaction of the embryo of *Sambucus* changes from alkaline to acid, but the endosperm remains alkaline. Both dry and germinating *Rubus* seeds are acid. A qualitative analysis of the ash of *Tilia* seeds showed iron, calcium, magnesium, potassium, and aluminium present. No tests were made for sodium.

Experimental data

Freshly harvested *Tilia* seeds with a moisture content of 10 per cent or less, or seeds kept in dry warm storage for several months, fail to germinate when placed on a moist substratum and kept at room temperature. This is true not only of seeds with coats intact, but for those with the coats chipped or entirely removed. Fungi and bacteria soon attack seeds with the coats broken and decay takes place in a few days. The percentage of water held by air-dry seeds is shown in table II. The seeds used for these determinations were dried in a partial vacuum at 80° C. until the weight was constant.

TABLE II

WATER CONTENT OF AIR-DRY *Tilia* SEEDS

Condition of seeds	Weight of air-dry seeds in gm.	Water loss in gm.	Percentage of water loss
Coats off.....	1.2754*	0.0788	6.17
Coats on.....	1.8559	0.1782	9.60
Coats on.....	2.1904	0.1574	7.18
Coats on.....	1.5024	0.1130	7.52

*Average of 4 duplicates.

The variations in the percentage of water lost by the seeds with coats on is due to the presence of seed coats which contained no endosperm and embryo. That the failure of air-dry *Tilia* seeds,

coats either on or off, to germinate is not due to an inability to absorb water is indicated by table III. The data given in this table were obtained by soaking seeds in distilled water at room temperature until they had come to constant weight. Here again the

TABLE III

WATER-HOLDING CAPACITY OF AIR-DRY *Tilia* SEEDS

Condition of seeds	Weight of air-dry seeds in gm.	Water absorbed in gm.	Percentage of water absorbed
Coats off	1.2848*	1.2071	93.95
Coats on	2.1540	0.7841	36.40
Coats on	1.7842	0.4146	23.24
Coats on	2.1198	0.4804	22.66
Coats chipped	1.4963	1.5020	100.38
Coats chipped	1.5040	1.5717	104.50
Coats chipped	1.9590	1.9185	97.93

*Average of 4 duplicates.

variations in the percentage of water absorbed are in part due to the presence of seed coats which contain no endosperm and embryo. Even with the coats chipped it is not always possible to eliminate all empty coats or defective seeds. The fact that the coats interfere with water absorption to a considerable extent is clearly shown in the table. The fact that seeds with coats removed or chipped, however, and with a moisture content approximately equal to their air-dry weight will not germinate when placed on a moist substratum at room temperature, is sufficient proof that water absorption is not the only limiting factor to growth.

That seeds that have been stored in the air-dry condition when the seed coats are intact can be forced to germinate is shown by the following experiment. Approximately 7000 seeds (200 gm.) of the 1916 crop, with pericarps removed and coats chipped, were placed on moist cotton in large Petri dishes and kept at 4–6° C. from March 24, 1917, to June 10, 1917, a total of 78 days. At the end of that time and before being transferred to a higher temperature, several hundreds showed the hypocotyl protruding from the endosperm for 1.5–2.5 cm. Of these, 100 were planted in soil in the greenhouse and 71 per cent produced seedlings. A second lot of 100 seeds was planted in soil out of doors, and 64 per cent

produced seedlings. Two lots of 500 each were selected from the seeds in which the hypocotyl was still inclosed within the endosperm. These were planted in soil in the greenhouse and in the garden and gave 20 and 25 per cent germination respectively. All seeds not planted were again placed in cold storage. Twelve days later 400 with hypocotyls protruding from the endosperm were planted in soil in the greenhouse. Of these, 348, or 87 per cent, produced seedlings within a week. By July 24, 1666 of these 7000 cold storage seeds had germinated at a low temperature. Of the ungerminated seeds 100 placed on moist cotton at room temperature gave 31 per cent germination in one week. The roots of these were short and thick and showed a great tendency to coil. At the same time air-dry seeds which had been stored at room temperature, when placed in soil or on moist cotton, decayed. Seeds kept in cold storage showed for the first few days a great tendency to mold, so that it was necessary to sterilize them with a 3 per cent solution of hydrogen peroxide for 1 hour on two separate occasions. With longer storage an immunity toward fungi is established, and although the coats may be covered with a thick layer of mycelia the endosperm and embryo are not attacked. Sections of the seeds examined under the microscope failed to show any hyphae present within the living tissue. On November 6, 1917, 6 cultures of 50 seeds each of both the 1916 and 1917 crops were placed in moist storage at 0–2° C., where they were allowed to remain for 140 days. At the end of that time no germination had taken place, which is in direct contrast with the result obtained in 1916 with seeds stored at 4–6° C. The failure to obtain germination here is interpreted as being due to the use of too low a temperature. The assumption that the exposure to this temperature was too long will hardly explain the results obtained, since if the temperature were not too low germination should begin as soon as the after-ripening process is complete. The results given in table IV, showing the percentage of germination obtained when these seeds were transferred to a temperature of 10–12° C., indicate that the storage temperature and not the length of exposure to it is the limiting factor.

This conclusion is strengthened further by the following experiment. Unfortunately no count of the number of seeds germinated

was made, as the experiment was used primarily for a different purpose. Approximately 1000 seeds of each of the 2 crops, stored under the same conditions as those indicated in table IV, showed no

TABLE IV

SEEDS OF *Tilia* STORED AT 0–2°C. FOR 140 DAYS; THEN AT 10–12°C.

NUMBER OF CULTURE	PERCENTAGE OF GERMINATION AFTER			
	12 days at 10–12° C.		19 days at 10–12° C.	
	1916 seeds	1917 seeds	1916 seeds	1917 seeds
1	66	24	74	28
2	68	20	72	24
3	70	12	80	18
4	74	34	82	34
5	70	26	76	30
6	19	22	56	30

germination after 140 days at a low temperature. When brought to the higher temperature the 1916 seeds germinated vigorously and in large numbers for the first 12 days and until the hypocotyls were 2–3 cm. long. From this point on no development took place and the seedlings gradually died. Here a temperature of 10–12° C. seems to be too low for continued growth. The 1917 seeds germinated much less vigorously, in fewer numbers, and only a few developed hypocotyls 2 cm. long. Comparing the results obtained in 1916 with those obtained in 1917, it is seen that the seeds after-ripen and germinate at temperatures slightly above freezing. DAVIS and ROSE (7) working with *Crataegus* found that after-ripening takes place most rapidly at 3–6° C., and that temperatures considerably higher are more favorable for germination and growth. At 0–2° C. *Tilia* seeds after-ripen but do not germinate. At 4–6° C. after-ripening and germination both take place, the latter taking considerable time. After-ripened seeds germinate poorly at room temperature. Once germination has begun at the low temperature, growth is best at temperatures above 12° C. The germination of *Tilia* seeds depends, therefore, upon the proper regulation of the temperature, and can be accomplished by a period of after-ripening in moist storage at 0–2° C., followed by a sojourn of 2 or 3 weeks at 10–12° C. until germination is well under way,

and finally by a transfer to a still higher temperature in order to permit vigorous growth. These conclusions are drawn from the facts that (1) seeds after-ripened at 0–2° C. did not germinate until transferred to a temperature of 10–12° C.; (2) although germination began at the higher temperature, growth soon ceased; and (3) seeds which had been after-ripened and which had begun to germinate at 4–6° C. grew well when transferred to soil in the greenhouse. Table IV suggests that one-year old seeds are better than fresh, but additional data upon this point are desirable. A nurseryman with many years' experience in the growing of trees and shrubs states that if *Tilia* seeds are allowed to become dry between the time of maturing and the time of layering a low percentage of germination results. On the other hand, if a high moisture content is maintained during this period no difficulty in germination is encountered. Up to the present time the author has been unable to obtain seeds which at the time of gathering had a moisture content of more than 10 per cent, and it seems probable that the water content of *Tilia* seeds is generally low at harvest time. While these seeds do not after-ripen to any considerable degree in air-dry storage, those that have been in the air-dry condition for a year after-ripen perfectly when put in a moist germinator at a low temperature. There seems to be no injury, therefore, even from protracted air-dry storage. No discussion is necessary to show that field conditions are not those most favorable for the obtaining of high percentages of germination. Neither does the nurseryman, when layering seeds, control the temperatures to the extent necessary to secure maximum results.

Hydrogen ion concentration.—The determinations of the hydrogen ion concentrations were made with the hydrogen electrode. Twenty seeds were pulverized in a mortar, and, except in instances to be noted later, 25 cc. of water added. The temperature varied from 27 to 33° C., but in every case the necessary correction was made. The determinations were made upon the seeds in the unafter-ripened condition, after-ripened but not germinated, with hypocotyl 2 mm. to 5 mm. long, and with hypocotyl 0.5 cm. to 2 cm. long.

Eckerson (8) has already shown that the acidity of the hypocotyl of *Crataegus* increases as after-ripening progresses. Her

determinations were made by the titration method with phenophthalein as an indicator. The P_H of seeds with the hypocotyls 0.5 cm. to 2 cm. long is approximately 4 times as great as that of the unafter-ripened seeds. While this is not as great an increase as that found by ECKERSON, it may be due to the fact that her determinations were made upon the dormant organ alone, while here the whole seed was used, or to differences between the two kinds of seeds. Determinations made by the titration method would also probably give values much higher than those obtained by the hydrogen elctrode.

Table V shows that the weight of the seeds increases as after-ripening progresses. This is not due to an increase in dry weight,

TABLE V

CONCENTRATION OF HYDROGEN ION OF *Tilia* SEEDS IN DIFFERENT STAGES OF AFTER-RIPENING

Condition of seeds	Weight in gm.	P_H
1 Air-dry	0.384	2.24×10^{-7}
2 Air-dry	0.443	2.00×10^{-7}
3 Air-dry	0.379	2.58×10^{-7}
4 Air-dry	0.379	2.40×10^{-7}
5 After-ripened	0.714	3.24×10^{-7}
6 After-ripened	0.752	3.02×10^{-7}
7 With hypocotyls 5 mm.	0.943	6.76×10^{-7}
8 With hypocotyls 5 mm.	0.932	5.75×10^{-7}
9 With hypocotyls 5 mm.	0.946	7.59×10^{-7}
10 With hypocotyls 0.5–2 cm.	1.553	1.18×10^{-6}
11 With hypocotyls 0.5–2 cm.	1.638	1.10×10^{-6}
12*With hypocotyls 0.5–2 cm.	1.578	9.33×10^{-7}
13*With hypocotyls 0.5–2 cm.	1.709	9.33×10^{-7}
14†With hypocotyls 0.5–2 cm.	1.450	1.05×10^{-6}
15†With hypocotyls 0.5–2 cm.	1.571	1.05×10^{-6}
16 After-ripened at room temperature (10 days)	0.689	2.51×10^{-7}
17 After-ripened at room temperature (10 days)	0.642	2.51×10^{-7}

*50 cc. of water used.

†100 cc. of water used; 25 cc. of water used for all others.

since no photosynthesis has taken place, but to the large amount of water absorbed. ECKERSON (8) likewise observed an increased water-holding capacity for the hypocotyl of *Crataegus* as after-ripening progressed. Of greater significance in this connection is the fact that, at least for the most advanced stage of after-ripening, variations in the amount of water used with the sample had little

effect upon the hydrogen ion concentration. With samples 10 and 11, 25 cc. of water were used, with samples 12 and 13, 50 cc., and with samples 14 and 15, 100 cc. Although the variation of P_H is considerable, it is by no means as great as that of the amount of water used, nor is it in the same direction. That the degree of dilution has no effect upon the P_H suggests the presence of buffer salts, formed by the action of fatty acids produced during germination and the constituents of the ash already mentioned.

After-ripened seeds similar to those used in samples 5 and 6, which had failed to germinate when kept at room temperature for 10 days, gave a P_H corresponding very closely to that shown by unafter-ripened seeds. This suggests that after-ripening is a reversible process, a fact to which CROCKER (5) has called attention, and that a decrease in acidity may lead to secondary dormancy.

TITRATABLE ACID.—Determinations of the titratable acid were made upon dry, after-ripened, and germinating seeds. For each determination the seeds were ground in a mortar with 10 cc. of water and titrated with N/10 sodium hydroxide with phenophthalein as an indicator. Titrations were made with freshly prepared samples and with others which had been allowed to stand for 48 hours. To the latter were added 10 drops of toluol and 0.5 cc. of N/10 hydrochloric acid. Table VI shows the number of cubic centimeters of N/10 sodium hydroxide necessary to neutralize the free acid in each sample. The figures are the average of duplicate determinations. Corrections have been made for the acid added.

TABLE VI

Condition of seeds	Fresh samples	After 48 hours	Percentage of increase
Dry	0.41	0.87	112.2
After-ripened	0.45	1.87	315.5
Germinating	1.18	2.80	137.2

While the amount of acid present is greatest in germinating seeds, it is seen that after autodigesting 48 hours the greatest percentage of increase over the freshly prepared samples is in seeds well after-ripened. Here is shown the fact that the after-ripened

seeds have a great power of increasing their alkali absorption, which may be due to lipase activity.

CATALASE.—Determinations of catalase activity of dry, after-ripened, and germinating seeds were made by means of APPLEMAN'S apparatus (1). The samples, ground in a mortar, were all reduced to the same degree of fineness by rubbing them through bolting cloth. The catalase determinations were made at 25° C. To 5 cc. of water containing 0.02 gm. of pulverized seed material was added 5 cc. of Oakland dioxygen and the amount of oxygen released was measured after 1, 2, 3, and 5 minutes of activity. APPLEMAN has pointed out that small amounts of acid greatly reduce or entirely destroy catalase activity. In order to remove this possible source of error the Oakland dioxygen used was neutralized by the addition of N/10 NaOH, or an excess of $CaCO_3$ was added to the meal. The data given in table VII are the averages of duplicate determinations. They show that dry, after-ripened, and germinating seeds, in the order named, exhibit increasing catalase activity. ECKERSON (8), employing microchemical methods, arrived at similar conclusions for seeds of *Crataegus*.

TABLE VII

CONDITION OF SEEDS	REACTION OF REAGENT	OXYGEN IN CC. LIBERATED AFTER			
		1 minute	2 minutes	3 minutes	5 minutes
1. Dry seeds	Neutralized*	2.5	4.2	5.25	7.0
2. After-ripened (dried 2 days)	Neutralized*	7.1	11.8	15.4	21.5
3. After-ripened (dried 2 days)	With $CaCO_3$	6.8	11.9	14.8	21.05
4. After-ripened (not dried)	With $CaCO_3$	6.75	11.3	15.05	20.75
5. Germinating	With $CaCO_3$	19.4	27.86	31.5	37.03

*0.80 cc. N/10 NaOH to neutralize 25 cc. dioxygen.

Drying after-ripened seeds for 2 days at room temperature has no effect on the amount of oxygen liberated, as is shown by comparison of samples 3 and 4.

Further evidence for the effect of the acid of the dioxygen upon catalase activity is shown in table VIII. Determinations made with after-ripened seeds not dried and with germinating seeds gave similar results. A comparison of the last 2 determinations show

that the neutralization of dioxygen or the addition of $CaCO_3$ is sufficient to eliminate any error due to the acidity of the reagent or the meal.

TABLE VIII

EFFECT OF REACTION OF SOLUTION UPON AMOUNT OF OXYGEN LIBERATED FROM DIOXYGEN BY *Tilia* SEEDS

CONDITION OF SEEDS	REACTION OF REAGENT	OXYGEN IN CC. LIBERATED AFTER			
		1 minute	2 minutes	3 minutes	5 minutes
After-ripened (dried 2 days)....	Not neutralized	2.1	3.1	3.6	4.3
After-ripened (dried 2 days)....	Neutralized	7.1	11.8	15.4	21.5
After-ripened (dried 2 days)....	With $CaCO_3$	6.8	11.9	14.8	21.05

OXIDASE ACTIVITY.—The determinations of oxidase activity were made on dry, after-ripened, and germinating seeds in BUNZELL'S (4) simplified apparatus with pyrogallol as the reagent. The material used, except in the case of the dried seeds, had been after-ripened at 0–2° C. for 140 days and then kept at 10–12° C. until a large percentage of the seeds had begun to germinate. After being dried in a vacuum over lime for 3 days at room temperature the seeds were ground in a mortar and the determinations made on 0.02 gm. of meal. Table IX shows the readings in centimeters of mercury after 3 hours and after 20.5 hours.

TABLE IX

OXIDASE ACTIVITY OF DRY, AFTER-RIPENED, AND GERMINATING SEEDS OF *Tilia*

Time	Dry seeds	After-ripened	Hypocotyls 1–5 mm. long	Hypocotyls 0.5–2 cm. long
After 3 hours.....	0.52	1.03	2.01	1.39
After 3 hours.....	0.53	1.10	1.92	1.52
After 20.5 hours.....	0.67	1.52	2.57	2.42
After 20.5 hours.....	0.68	1.67	2.52	2.07

During the experiment the temperature averaged 31.3° C. with a variation of ±0.1 of a degree. Variations in the volume of air in the tubes due to this slight variation in temperature have been corrected by means of check tubes containing water only. The

results show that the oxidase activity rises with after-ripening and germination. Once germination has begun, no increase is to be noted.

Discussion.—The results obtained show that the dormancy exhibited by the seeds of *Tilia* is not due to any property of the seed coat, although that structure may serve to lengthen the dormant period, but is to be ascribed to conditions obtaining within the endosperm or the embryo or both. In this respect *Tilia* resembles *Crataegus*, and the conditions necessary for after-ripening and germinating of the former are very similar to those required by the latter. Even with these conditions well known and various differences between dormant and after-ripened seeds clearly shown, it is still impossible to define the term after-ripening in anything more than general terms. The similarity of *Tilia* and *Crataegus*, with respect to the conditions necessary for after-ripening, does not permit one to conclude that the process in the two is the same. In any case after-ripening is not to be attributed to a change in any one condition, but to a series of changes which may vary for each individual case. Dormancy is to be looked upon, perhaps, as a condition of equilibrium in a series of chemical reactions; after-ripening as a displacement of this condition. Why low temperatures are effective in causing these changes and why the range of effective temperatures is so narrow are questions still to be answered.

Sambucus

Kinzel (15) states that for *Sambucus nigra* freezing for 2 winters is sufficient to bring only 39 per cent of the seeds to germination. Even longer freezing is necessary for the seeds of *S. racemosus*. Results obtained by the writer in experiments to be described are very similar to those given by Kinzel, and show that in neither case have the conditions necessary for germination been even approximately determined.

Nurserymen claim that layering results in almost perfect germination if the seeds are not allowed to become dry between the time of maturing and the time of layering. Air-dry seeds are considered worthless. These statements are in a large measure

confirmed by the following experiments, although sufficient data are not yet available to warrant a final statement.

Seeds removed from berries and allowed to dry at room temperature for 2 days failed to germinate within 2 weeks when placed on moist cotton, although they never contained less than 22 per cent of moisture. Fresh seeds on moist cotton kept at 4–6°, 0–2°, or 8° C. have never given more than 20 per cent germination when placed at room temperature or above. Air-dry seeds have given no better results. Although these seeds were kept at the low temperature for not less than 2 months, a longer period may be necessary. The experiments show that failure to germinate is not entirely due to injury resulting from drying, although that may be one of the determining factors. Neither is it to be attributed to inability of air-dry seeds to absorb water, since the quantity taken up in 48 hours by seeds with coats intact is equal to 38.55 per cent of their air-dry weight, while seeds with coats punctured absorb 39.16 per cent. Air-dry seeds contain approximately 6 per cent of water.

The effect of layering is shown by the following experiments, in which the number of seeds used for the 1916 crop was 1000 and for the 1917 crop 5000. Two lots of air-dry seeds of the 1916 crop were mixed with soil. One lot was kept at 15–20° C., the other out of doors over winter. In spring the percentages of germination were 8 and 44 respectively. Fresh seed of the 1917 crop, which had not been permitted to become dry when treated in the same way, gave 51 per cent and 77 per cent respectively. Air-dry seeds of the 1916 crop one year old failed to show any germination. Loss of water seems to be accompanied by a reduction in vitality.

Air-dry seeds gathered on October 14, 1916, were treated within 30 days with weak solutions of a large number of acids, bases, and salts. The acids used were malic, citric, tartaric, acetic, and butyric; the bases, potassium hydroxide, ammonium hydroxide, and sodium hydroxide; and the salts, sodium sulphate, nickel sulphate, ammonium sulphate, zinc sulphate, potassium sulphate, potassium nitrate, sodium nitrate, cobalt nitrate, ammonium nitrate, calcium chloride, sodium chloride, barium chloride, and potassium thiocyanate. The dilutions of the acids were N/200

and N/400; of the bases, N/1000, N/2500, N/5000, and N/10,000; and of the salts, N/20 and N/200. The number of perfect seeds in the cultures varied from 43 to 96. In only 4 cases was the number below 60, and the average was 75. This variation is due to the presence of empty seed coats which could not be distinguished from the perfect seeds until they had taken up a considerable quantity of water. It was later found possible to candle the seeds and thus eliminate the majority of the empty coats. The candling was done by means of an incandescent light supported below a glass plate upon which the seeds were placed. Between the light and the plate was placed a vessel of water to prevent undue heating. The seeds were placed in 20 cc. test tubes containing the solutions and allowed to soak for 24 hours. At the end of that time the solutions were drawn off and the seeds distributed over the moist walls of the test tubes, which were then plugged with cotton and kept at a temperature varying from 4 to 23° C. As soon as the seeds began to show signs of germination, they were removed from the tubes and placed in Petri dishes on moist cotton and kept at room temperature. Germination was slow, in the majority of cases extending over a period of 3 months. In the case of acetic acid, N/400, 58 per cent of the seeds germinated at the end of 176 days. The acids other than acetic showed little effect. The length of time over which bases can have any effect must be short, since in dilute solutions they are soon neutralized by the carbon dioxide of the air and that produced by the seeds. The cultures which showed germinations equal to or better than the checks are listed in table X.

In order to test the effect of constant low temperature upon seeds soaked in solution of various chemicals, a second set of cultures was prepared in the manner already described and kept at 4–6° C. for 63 days. At the end of that time the tubes were placed at room temperature. To the list of substances used in the preceding experiment were added potassium citrate, potassium tartrate, potassium acetate, potassium chlorate, ammonium nitrate, potassium iodide, lithium chloride, ammonium chloride, magnesium chloride, sodium nitrite, and dipotassium phosphate, and also hydrochloric acid and sulphuric acid. The concentrations of the mineral acids were N/1000, N/2500, N/5000, N/10,000, and of the

salts N/20, N/200, and N/1000. Germination began 4 days after the cultures were placed at room temperature and continued for 18 days. At the end of that time in practically all of the cultures, in addition to the seeds which had germinated, others were found

TABLE X

Sambucus SEEDS IN DILUTIONS OF ACIDS, BASES, AND SALTS; TEMPERATURE 4–23° C.

Substance	Normality of solution	Number of seeds	Percentage of germination
Distilled water	.....	78	12
Distilled water	.....	85	10
Distilled water	.....	68	10
Distilled water	.....	83	13
Acetic acid	N/200	79	18
Acetic acid	N/400	72	28
Malic acid	N/400	75	15
NH_4OH	N/2500	77	18
NaOH	N/1000	88	19
NaOH	N/2500	70	17
$(NH_4)_2SO_4$	N/20	75	28
$(NH_4)_2SO_4$	N/200	67	15
$ZnSO_4$	N/20	50	22
KNO_3	N/200	80	30
$NaNO_3$	N/20	46	19
$NaNO_3$	N/200	72	30
$CoNO_3$	N/200	59	71
KCNS	N/200	66	31

with the seed coat ruptured, but showing no sign of growth. All cultures in which a forcing effect of the solution is indicated by the germination of 20 per cent or more of the seeds are listed in table XI.

Out of 13 other substances not given in the table, 5 showed results equal to or better than the average of the checks in at least one dilution. The nitrates and sulphates are again found among the more effective substances. So far as the nitrogen compounds are concerned, these results agree with those of GASSNER (10) for seeds of *Chloris ciliata*.

Potassium nitrate, mercuric chloride, and potassium iodide used in connection with alternating temperatures had even less forcing effect than the substances given in table XI. The concentrations used were for potassium nitrate N/20, N/100, N/200, N/500,

N/1000, N/2000; for mercuric chloride N/400, N/1000, N/2000, N/4000, N/10,000; and for potassium iodide N/20, N/100, N/500, N/1000, N/2000. Three sets of cultures were set up in duplicate.

TABLE XI

Sambucus SEEDS IN ACIDS, BASES, AND SALTS

Substance	Normality of solution	Number of seeds	Percentage of germination	Percentage of seeds with ruptured coats	Total percentage of seeds affected
HCl	N/5000	65	17	6	23
H_2SO_4	N/2500	66	18	10	28
H_2SO_3	N/10,000	63	15	6	21
NH_4OH	N/1000	89	23	16	39
NH_4OH	N/5000	96	13	8	21
$C_6H_8O_9$	N/400	101	21	4	25
KNO_3	N/20	78	28	25	53
KNO_3	N/200	77	13	18	31
KNO_3	N/1000	86	24	8	32
$CoNO_3$	N/200	92	4	44	48
$CoNO_3$	N/1000	87	24	23	47
NH_4NO_3	N/200	108	14	39	53
NH_4NO_3	N/1000	56	14	12	26
$NaNO_3$	N/20	96	20	18	38
$NaNO_3$	N/200	83	33	20	53
$NaNO_2$	N/200	72	25	26	51
$NaNO_2$	N/1000	65	9	18	27
Na_2SO_4	N/200	94	10	10	20
$NiSO_4$	N/20	84	10	10	20
$NiSO_4$	N/200	86	7	34	41
$NiSO_4$	N/1000	76	15	10	25
$(NH_4)_2SO_4$	N/200	85	5	37	42
$ZnSO_4$	N/20	86	41	1	42
KCL	N/20	80	0	22	22
LiCl	N/1000	80	0	22	22
NaCl	N/200	89	1	37	38
NH_4Cl	N/20	97	2	24	26
NH_4Cl	N/200	84	13	15	28
Potassium citrate	N/20	84	0	21	21
Potassium citrate	N/1000	95	7	37	44
$KClO_3$	N/1000	81	9	18	27
KI	N/20	89	10	33	43
KCNS	N/200	90	9	12	21
K_2HPO_4	N/20	83	1	24	25
K_2HPO_4	N/1000	55	0	34	34
Distilled water	.....	70	0	7	7
Distilled water	.....	85	8	7	17
Distilled water	.....	109	1	14	15

One set was kept at 20° C. and a second at 30° C. The third set was kept at 20° C. for 18 hours and at 30° C. for 6 out of every 24 hours. The air in the tubes was changed every second day. The

duration of the experiment was 38 days. At the end of that time the only germinations obtained were those in the potassium nitrate, and in no case did these exceed 4 per cent. The seeds in the stronger mercuric chloride solutions were killed.

The rôle played by the coat in the behavior of the seeds has not been determined. Of naked embryos placed on moist cotton 32 per cent developed chlorophyll within a week, formed the hypocotyl arch, and attained a length of 5–10 mm. Naked embryos previously soaked in dilutions of hydrochloric acid and butyric acid and then placed on moist cotton gave no better results.

Seeds treated with concentrated sulphuric acid for 4–60 minutes and then kept under various conditions in regard to light, temperature, and oxygen pressure have never given over 20 per cent germination. A slight forcing effect by low concentrations of sulphuric acid was observed on seeds previously treated with concentrated sulphuric acid for 2–14 minutes and kept in the light at room temperature. Seeds immersed for 5 minutes in water at 40° C. in 55 days gave 25 per cent germination. Reheated at the same temperature for 3 minutes, 33 per cent germinated after 40 days. Longer heating at 40° C. or up to 70° C. gave lower percentages of germination. Untreated seeds gave no germination in the same length of time.

These results emphasize the following facts concerning the conditions necessary for the germination of *Sambucus* seeds: (1) air-dry seeds with a moisture content of 6 per cent or fresh seeds with a moisture content of 22 per cent will not germinate when placed on a moist substratum at room temperature; (2) this is not due entirely to injury resulting from drying, although that may be one of the determining factors; (3) air-dry seeds are able to absorb water to the extent of approximately 40 per cent of their air-dry weight, indicating that failure to germinate is not due to lack of water; (4) the effect of chemicals upon air-dry seeds is not marked, a slight forcing effect of several acids, bases, and salts has been observed, among which substances are found nitrates and sulphates; (5) the rôle played by the coat in the behavior of the seed has not been fully determined; (6) a slight forcing effect by low concentrations of sulphuric acid and by water at 40° C. has been observed;

(7) seeds remaining in contact with moist soil out of doors over winter gave 77 per cent of germination the next spring; whether this result is due to the low temperature, to certain constituents of the soil, or to a combination of these or other factors one cannot say.

The results obtained by KINZEL (15), together with those just summarized, show that as yet the conditions necessary for the germination of *Sambucus* seeds are not fully determined. To permit the water content of the seeds to fall below an undetermined critical point may lessen their viability. However, that some other condition or combination of conditions is responsible for the low percentages of germination must not be overlooked. KINZEL'S suggestion that prolonged freezing is necessary should be given due consideration.

RUBUS

Seed fruits of *Rubus Idaeus*, like the seeds of the 2 species already discussed, fail to germinate when placed on a moist substratum. It was determined that this is not due to an immature condition of the embryo. If the pericarp is left intact all treatments with low concentrations of acids, bases, and salts, immersion in warm water, cold storage, exposure to increased oxygen pressure, or to ether vapor, freezing and thawing, and injection with water under pressure are ineffective.

When buried in the soil at 15–20° C. or out of doors over winter, a low percentage of germination takes place if the seeds are kept moist. Two lots of 720 viable seeds buried for 140 days under these conditions gave respectively 40 per cent and 20 per cent germination. Of 2 similar lots of seeds buried in tightly stoppered bottles, one at constant, the other at varying temperatures, none germinated when planted in the soil in the greenhouse. That these results are not due to injury resulting from drying or to inability to absorb water is indicated in table XII. The removal of the endocarp was accomplished by soaking the seeds in concentrated sulphuric acid for approximately 2 hours. Following this treatment the seeds were washed quickly in a large amount of running water to prevent heating, then immersed in a 5 per cent solution of sodium bicarbonate to neutralize the remaining acid,

and finally rinsed in running water for 15 or 20 minutes. The carbonized endocarp was removed by rubbing the treated seed on filter paper. The selection of perfect seeds was now an easy matter.

Table XII shows that the water-absorbing power for the seeds with the endocarp removed is 36–37 per cent of their air-dry weight, while that for the seeds with the endocarp intact reaches

TABLE XII

WATER CONTENT AND WATER HOLDING CAPACITY OF *Rubus* SEEDS

Condition of seeds	Weight of air-dry seeds in gm.	Weight of seeds dried in vacuum at 75° C.	Percentage of water in air-dried seeds	Weight of soaked seeds in gm.	Water absorbed by air-dry seeds in gm.	Percentage of water absorbed by air-dry seeds
Endocarp removed .	0.6686	0.5842	12.62*	0.9120	0.2434	36.40*
Endocarp removed .	0.6864	0.6009	12.45	0.9414	0.2550	37.15
Endocarp intact....	1.0464	0.9328	10.85	1.5053	0.4589	43.85
Endocarp intact....	2.0960	1.8680	10.87	3.0080	0.9120	43.51

* On basis of air-dry weight.

almost 44 per cent of their air-dry weight. From this it follows that the water absorbing power of the endocarp is greater than that of the seed with the endocarp removed. There is no evidence to show that the endocarp possesses any structure which would prevent the water absorbed by it from being passed on to the seed. Although seeds with the endocarp intact will not germinate, when that structure is removed by means of the sulphuric acid treatment germination takes place within a few days, as is shown in table XIII.

The greater amount of the germination takes place between the fourth and tenth days. In seeds germinating after the tenth or twelfth day, growth is usually slow and the seedlings are weak. Failure to secure 100 per cent germination is due to the fact that during the removal of the carbonized endocarp in almost every case the seed coat is ruptured and the endosperm exposed to the attacks of bacteria and fungi. With the carbonized endocarp intact, uncertainty as to the extent to which the acid had penetrated and the inability to determine the number of fruits containing viable embryos lead to greater error than that occasioned by the attacks of the bacteria and fungi.

MÜLLER (20) has recently pointed out that in various seeds that germinate readily the outward pressure of the contents at the time of rupture was but slightly greater than the breaking strength of the water-saturated coat, and CROCKER and DAVIS (6) have found that seeds of *Alisma* are held in a dormant condition because the force of the expanding contents is not sufficient to rupture the coats.

TABLE XIII

SEEDS OF *Rubus Idaeus* WITH ENDOCARP REMOVED; 100 SEEDS PER CULTURE; TEMPERATURE 18–23° C.

TREATED WITH ACID	PERCENTAGE OF GERMINATION AFTER				
	4 days	6 days	8 days	10 days	20 days
May 4	.....	.....	70	84	96
May 13	2	45	52	63	70
May 13	32	48	53	61	61
May 13	24	46	.....	55	55
May 24	.....	50	73	83	88
May 24	.....	20	63	77	84
May 24	.....	22	61	.....	80
May 24	.....	40	78	86	88
May 24*	.....	46	78	86	93
May 24*	.....	57	77	82	89
June 9	.....	50	.....	86	95

* In darkness.

Failure to absorb water is not the limiting factor, since both reach saturation after about 5 hours' soaking. Two facts indicate that *Rubus* seeds belong in the same class with *Alisma*. In the first place they germinate readily once the endocarp is removed, and in the second place even with the endocarp intact they absorb water readily. Occasionally ungerminated seeds with the endocarp removed have been found which when examined closely show no break in the coat. This suggests that the inner pectinized layer of the coat may play a part in the delay, either by limiting water or oxygen absorption, or both. As already indicated, the removal of the carbonized endocarp resulted in the rupture of the coat in practically 100 per cent of the seeds. This renders extremely difficult the determination of the part played by that structure.

Table XIV shows that the substrata most favorable for germination of naked seed are cotton, filter paper, and quartz sand. An

inhibitory effect is shown by garden soil, clay, and greenhouse soil, the effect of the last named being greatest. These soils acidified gave no better results. Calcium carbonate used on filter paper or in sand to neutralize any acid present in the medium or remaining on the seeds after the sulphuric acid treatment had no inhibitory effect. Glass wool moistened with a boiling water extract or a cold water extract of greenhouse soil cut down the percentage of germination to less than 50. Moreover, the seedlings were weak, with enlarged and discolored roots. In many cases germination started, but the roots were killed as soon as they came in contact with the substratum. Bone meal had been added to the greenhouse soil and this probably accounts for the injurious effect of the soil and the extracts. As is shown in table XIV, soaking in water for 24 hours

TABLE XIV

EFFECT OF SUBSTRATUM UPON GERMINATION OF NAKED SEEDS OF *Rubus Idaeus;* 100 SEEDS PER CULTURE; TEMPERATURE 18–23° C.

SUBSTRATUM	PERCENTAGE OF GERMINATION AFTER				
	6 days	8 days	10 days	12 days	25 days
Filter paper	50	77	86	90	95
Filter paper with $CaCO_3$	31	84	87	90	92
Quartz sand	48	80	83	85	92
Quartz sand	42	83	88	89	93
Quartz sand with 5 per cent $CaCO_3$	43	78	80	.....	88
Greenhouse soil	.....	.....	10	13	25
Greenhouse soil	.....	.....	.....	1	1
Greenhouse soil, acid	.....	.....	.....	1	1
Hot water extract greenhouse soil	4	22	41	.....	43
Cold water extract greenhouse soil	5	25	48	.....	48
Garden soil	5	25	.....	29	29
Garden soil, acid	1	9	.....	10	10
Clay	.....	3	9	11	19

previous to planting in garden soil raises the percentage of germination to 55. On the other hand, soaked seeds planted on moist cotton gave 71 per cent as against 72 per cent for unsoaked seeds. Germination was at practically the same rate in the two cases. Seeds planted on 5 per cent agar gave almost as high percentages as those on 1 per cent. The results given in tables XIV and XV show that the water supply is not the limiting factor.

Seeds in the soil are more exposed to attacks by fungi than those on agar or cotton. Previous soaking shortens the time the seeds must lie in the soil before germination begins, and hence lessens the chance for infection. Unsoaked seeds placed on moist cotton

TABLE XV

EFFECT OF WATER SUPPLY UPON GERMINATION OF SEEDS OF *Rubus Idaeus;* 100 SEEDS PER CULTURE; TEMPERATURE 20–25° C.

SUBSTRATUM	PERCENTAGE OF GERMINATION AFTER				
	6 days	8 days	10 days	12 days	16 days
1 per cent agar*	37	49	70	70	70
1 per cent agar*	22	50	.....	62	62
2 per cent agar*	30	37	50	50	50
2 per cent agar*	40	64	.....	73	73
5 per cent agar*	35	42	60	.....	60
5 per cent agar*	32	52	.....	61	61
Soaked 24 hours, then in garden soil	12	36	47	50	55
Soaked 24 hours, then on moist cotton	43	64	70	71	71
Not soaked, on moist cotton	32	66	72	.....	72

* Average of 2 duplicate determinations.

absorb water easily, hence swell more rapidly than in the soil, and moreover are less liable to infection. Under these conditions soaking offers no advantage.

Summary

GENERAL.—Air-dry seeds of *Tilia americana, Sambucus canadensis,* and *Rubus Idaeus* do not germinate when placed on a moist substratum at room temperature. In no case does water absorption seem to be the limiting factor. Air-dry seeds planted in the soil over winter give low percentages of germination.

TILIA.—Seed coats are not the cause of dormancy, although they may serve to lengthen the dormant period. A state of dormancy exists in the endosperm or embryo, or both.

Seeds with coats removed after-ripen at temperatures slightly above freezing. At 0–2° C. seeds after-ripen, but do not germinate. At 4–6° C. both after-ripening and germination take place. Seeds after-ripened at 0–2° C. germinate readily at 10–12° C., but very poorly at room temperature. Once germination has begun growth proceeds best at temperatures above 12° C.

As after-ripening progresses the hydrogen ion concentration increases, as do also the water holding capacity and the oxidase and catalase activities.

The greatest amount of free acid is present in the germinating seeds. Autodigestion of pulverized seeds shows the greatest acid increase in the after-ripened ungerminated seeds. This is probably due to their high lipase activity.

SAMBUCUS.—As high as 77 per cent of germination was obtained by layering fresh seeds out of doors over winter.

No satisfactory forcing agent has yet been found. A slight forcing effect of several acids, bases, and salts has been observed. The best of these forcing agents are nitrates and sulphates.

Although *Sambucus* seeds are probably injured by drying, that is not the only factor to be considered, since freshly gathered seeds with a moisture content of 22 per cent will not germinate when placed on a moist substratum.

As yet it has been impossible to approximate perfect germination, and much still remains to be learned concerning the conditions necessary to reach it.

RUBUS.—Dormancy is probably due to the high breaking strength of the endocarp. Seeds treated with concentrated sulphuric acid for 2 hours, then thoroughly washed, germinate readily on cotton, filter paper, or quartz sand.

The optimum temperature for germination lies between 20° and 25° C. Seeds germinate equally well in light or darkness. Naked seeds germinate poorly in soil. This may be due to the action of fungi, bacteria, or to other causes as yet unknown.

As a practical method for the germination of *Rubus* seeds, if one is not to resort to layering, the writer suggests the following: The seeds should be removed from the pulp as completely as possible. If the berries are crushed and then thrown into water most of the pulp can be floated off. The pulp still clinging to the seeds may be removed by allowing fermentation in water to take place or by treating the seeds with a 5 per cent solution of sodium hydroxide for 15–20 minutes, after which they should be thoroughly washed in running water. It is essential to dry the seeds for at least 24 hours, or the treatment with concentrated sulphuric acid which follows

will result in heating. The seeds should be left in the acid for approximately 2 hours.

In order to obtain uniform results it is advisable to use a large excess of acid and to prevent the seeds from gathering in clumps or layers. Frequent stirring is essential. By rubbing a few of the seeds in the palm of the hand from time to time it is possible to determine when the entire endocarp on a majority of the seeds has been carbonized. When this point is reached the acid should be drained away and the seeds thrown into an excess of cold water. It is advisable to change the water frequently or to put the seeds in running water, where they should be left for at least 15 minutes. When they are removed from the water they should be treated with an excess of a 5 per cent solution of sodium bicarbonate until bubbles cease to rise, after which they may be washed in running water for 15 minutes.

In order to remove the carbonized endocarp the seeds may be placed on filter paper and rubbed under the fingers. It is impossible to remove the endocarp if it has been allowed to become dry following the last washing.

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Missouri State Fruit Experiment Station
Mountain Grove, Mo.

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NOTES ON AMERICAN WILLOWS. IV

SPECIES AND VARIETIES OF SECTION LONGIFOLIAE

CAMILLO SCHNEIDER

In my paper on Mexican willows (BOT. GAZ. 65:22. 1918) I have already dealt with some species of this well marked and entirely American section. In this article I intend to discuss all the members of this interesting group, which is, as M. S. BEBB (1891) and W. W. ROWLEE (1900) rightly stated, clearly defined from the other sections of the genus in both the New and the Old World. ANDERSSON (1858) was the first to recognize the close relationship of species like *S. sessilifolia* Nutt., *S. Hindsiana* Benth., and *S. taxifolia* Kth. to *S. longifolia* Muhl. Unfortunately he misunderstood most of the species described by NUTTALL, and therefore he did not give, even in 1868, a proper analysis of the forms of this section. In 1900 W. W. ROWLEE (Bull. Torr. Bot. Club 27:247) made an attempt to rehabilitate all of NUTTALL'S species, and described several new species and varieties from the southwest, especially from California. His interpretation of NUTTALL'S species, however, is not free from grave errors owing to the lack of sufficient type material. Later C. V. PIPER studied those types of NUTTALL which are preserved in the British Museum, and communicated his notes to C. R. BALL, who in 1915 (BOT. GAZ. 60:49) was able to identify *S. sessilifolia* and *S. fluviatilis* Nutt. I have not seen the types in the British Museum, but I have photographs of NUTTALL'S specimens of *S. exigua*, *S. macrostachya*, and *S. melanopsis* from the Herbarium of the Academy of Science at Philadelphia. Besides this I have also examined a few of NUTTALL'S willows at the Gray Herbarium, which also contains some cotypes of forms described by ANDERSSON. Photographs and fragments of ANDERSSON'S types from the Hookerian Herbarium at Kew are now in possession of the Arnold Arboretum, and Professor W. W. ROWLEE kindly sent me the types of his new species and forms so far as they are preserved in the Herbarium of Cornell

University. I wish to acknowledge here his courteous assistance, and to give the same acknowledgment to the curators of the Herbarium of the Geological Survey of Canada at Ottawa, of the Gray Herbarium, of the Herbarium of the Royal Gardens at Kew, of the Missouri Botanical Garden, of the New York Botanical Garden, of Stanford University, and of the U.S. National Herbarium for the loan of material representing the forms under discussion. For further material I am indebted to Miss ALICE EASTWOOD, San Francisco, California, Professor J. K. HENRY, Vancouver, B.C., Professor W. L. JEPSON, Berkeley, California, Mr. I. M. JOHNSTON, Upland, California, and Mr. J. C. NELSON, Salem, Oregon. I have also been able to go over the material of the Bebb Herbarium at the Field Museum, and am under obligation to Dr. C. F. MILLSPAUGH for what he has done to further my studies.

Sect. LONGIFOLIAE Andersson in Öfv. K. Vet.-Akad. Förh. **15**: 116. 1858; for further literature see SCHNEIDER in BOT. GAZ. **65**: 22. 1918.—Frutices mediocres (rariter parvi) vel alti arboresque, ramis densis caespitosis, cortice cinereo vel pl.m. brunnescente, ramulis elongatis virgatis brunneis vel purpureo-brunneis interdum nitidulis. Folia linearia, lanceolata, vel elliptico-oblonga, denticulata vel integerrima, nervis lateralibus satis distantibus, petiolis vulgo satis brevibus, stipulis saepe deficientibus vulgo parvis lanceolatis denticulatis. Amenta serotina vel primaria coetanea, pl.m. pedunculata vel ramos laterales normaliter foliatos saepe satis longos terminantia, singula vel ad 2–3 aggregata, pl.m. cylindrica, rarius ovalia; bracteae concolores, flavescentes, deciduae; flores masculi vulgo biglandulosi, diandri, filamentis liberis pilosis; feminei fere semper uniglandulosi, stylis nullis vel brevibus, stigmatibus bifidis laciniis linearibus vel brevibus; ovaria fructusve pilosi vel glabri, subsessiles vel pedicello glandulam usque duplo (rarius magis) superante instructi.

As already stated, the LONGIFOLIAE is an entirely American group, of which *S. taxifolia* var. *microphylla* ranges as far south as Guatemala, while a form of *S. longifolia* almost reaches the Arctic Circle in the Yukon Territory. From west to east the range of the group extends from the shores of the Pacific to those of the Atlantic, but it is not represented in southeastern United States from central

Virginia to Alabama and Florida. The center of its development is from California to Washington, Montana, and Texas.

Among the American willows the LONGIFOLIAE occupy an isolated position, and of the willows of the Old World it is difficult to say which can be taken for the nearest relatives of this group. I shall discuss this point later, and I can now only repeat that probably the forms of the sect. ALBAE Borr. might be regarded as rather closely related genetically to the LONGIFOLIAE.

In the following key it is recognized[1] that there are two rather well marked types in the group based on the form of the stigmas. In one, represented by *S. taxifolia* and *S. sessilifolia*, the lobes of the stigma are narrow and elongated, and in the older flowers mostly more or less revolute; while in the other group, the types of which are *S. exigua* and *S. longifolia*, the lobes are shorter and broader, not linear-lanceolate, the whole stigma often being quasi capitate. In some forms of *S. longifolia*, especially of var. *Wheeleri* from the northeast, the shape of the stigmas is rather intermediate. In the first group *S. taxifolia* is well distinguished from *S. sessilifolia* and its relatives by the short small aments, the small more or less globose anthers, and the small linear leaves; while *S. sessilifolia* and its varieties and *S. fluviatilis* have long cylindric aments, oblong-ellipsoid anthers, and longer, broader leaves. In the second group it is more difficult to separate the species because the main characters, glabrousness or pubescence of the ovaries and leaves, are more liable to variation. *S. melanopsis* with var. *Bolanderiana* represents a rather well marked type with glabrous ovaries, but in *S. exigua* as well as in *S. longifolia* we meet with forms of which the ovaries vary from densely pubescent to entirely glabrous. The

[1] It seems to be of interest to quote BEBB's opinion as to the possibility of a taxonomic arrangement of the forms of this section (BOT. GAZ. 16:104. 1891): "Clearly marked as are the outer limits of the group it presents no lines of cleavage within by which it can be satisfactorily divided. No natural characters are found to coincide with such assumed distinctions, for instance, the 'linear lobes of the stigma,' made prominent in the attempt to separate *S. sessilifolia*. Each portion after subdivision remains as heterogeneous as was before the aggregate group. It may be possible, by emphasizing first one character and then another, as these are found to predominate in the different forms, to designate a number of subspecies and varieties; but so bewildering and intangible is the reticulated intergrading that the difficulty of segregation seems only to be heightened by every fresh acquisition of the material."

pubescence of the leaves too is very changeable, and only in connection with other characters can it be used to separate certain species and varieties.

Clavis specierum

Amenta brevia, mascula 5–13 mm. longa et circ. 8 mm. crassa, feminea satis pauciflora, fructifera haud ultra 2:1.2 cm. magna; antherae minimae pl.m. globosae vel subglobosae, haud vel paullo longiores quam latae; stigmatum lobi lineares vel lineari-lanceolati, vulgo 4–6plo longiores quam lati; stylus nullus vel subnullus; ovaria sessilia vel brevissime pedicellata; bracteae vulgo satis late obovato-rhombicae, pl.m. acutae, praesertim extus satis dense villosae; folia minima vel parva, linearia vel lineari-lanceolata, 10–30:1.5–3.5 mm. magna, subtus semper pl.m. sericea, margine breviter denticulata vel subintegerrima . . . . 1. *S. taxifolia*

Amenta longiora vel antherae ellipticae, circ. $1\frac{1}{2}$–2plo longiores quam latae vel folia majora.

Stigmatum lobi lineares vel lineari-lanceolati, elongati, vulgo 4–5plo longiores quam lati, adulti pl.m. revoluti, stylo satis distincto iis breviore vel brevissimo fere semper bifido suffulti vel pl.m. sessiles; ovaria (saltem juniora) distincte sericea vel sericeo-villosula; folia novella semper utrinque pl.m. dense sericea vel sericeo-villosa.

Ramuli hornotini dense, etiam annotini pl.m. sericeo-villosi vel tomentelli; folia etiam adulta utrinque concoloria, canescentia, canoviridia vel viridescentia, semper pl.m. sericea vel sericeo-villosa, nervis primariss vix vel non visibilibus; ovaria semper satis dense sericeo-pilosa, sessilia vel subsessilia, pedicello fructuum quam glandula plus quam 2plo breviore; bracteae rarius extus versus apicem glabrescentes (confer etiam 4. *S. Parishianam*).

Folia ramulorum fertilium lineari- vel anguste lanceolata, fere semper distincte integerrima, etiam majora vix ad 8 mm. lata, apice pl.m. sensim acuminata, basi acuta, in petiolum satis distinctum attenuata, stipulis fere semper nullis, vel folia maxima majora, 6–8 cm. longa et ultra 8 mm. lata; amenta mascula 1.5–3 cm. longa et 5–6 (rarius 8) mm.

crassa, feminea fructifera 2–4 (–6): 0.8–1 cm. magna, ovaria (fructusque) sessilia vel subsessilia.

Folia fere semper lineari- vel anguste lanceolata et vulgo integerrima, stigmata semper satis elongata et pl.m. revoluta..... 2b. *S. sessilifolia* var. *Hindsiana*

Folia fere semper remote denticulata, interdum late lanceolata; stigmata breviora, paullo curvata et magis sessilia..... 2c. *S. sessilifolia* var. *leucodendroides*

Folia ramulorum fertilium (anguste vel) late lanceolata vel elliptico-lanceolata, majora 8–15(–17) mm. lata, saepe (saltem ad apicem) pl.m. distincte subspinuloso-denticulata, interdum paene sessilia, stipulis saepe pl.m. evolutis; amenta mascula 3–4.5 cm. longa et circ. 7 mm. crassa, feminea fructifera 4–6(–10) cm.: 8–10 mm. magna, ovaria (fructusque) subsessilia vel brevissime pedicellata

2. *S. sessilifolia*

Ramuli tantum novelli satis dense sericeo-tomentelli, jam hornotini glabrescentes vel glabriusculi vel folia adultiora satis glabra subdiscoloria, vel ovaria fructusque glabri vel subglabri (confer etiam var. *Wheeleri* sub 8. *S. longifolia*).

Folia anguste lanceolata ellipticave, interdum oblanceolata, apice pl.m. acuminata, basi acuta, distincte petiolata, stipulis saepe evolutis, adultiora superne intense viridia, subtus interdum subglaucescentia, satis glabrescentia vel tenuissime sericeo-pilosa, nervis etiam secundariis utrinque pl.m. visibilibus, ramulorum fertilium 7–14 mm. lata; ovaria initio pl.m. sericea vel sericeo-villosa, matura vulgo fere tota glabrescentia, subsessilia, pedicello fructuum glandula sicca interdum subaequilongo, bracteae fere semper extus versus apicem glabrescentes interdum basi excepta glabra..... 3. *S. fluviatilis*

Folia anguste linearia ad lineari-lanceolata, 1.5–5 (–8) mm. lata, utrinque pl.m. dense adpresse sericea; ovaria pl.m. sericea vel fere glabra, fructus partim pilosi vel glabri sed pedicello brevissimo vulgo piloso..... 4. *S. Parishiana*

Stigmatum lobi lanceolati vel elliptici, satis breves, saepissime 2–3plo longiores quam lati, adulti ut videtur nunquam distincte

revoluti, stylo nullo vel brevissimo non bifido suffulti, ovaria sericea vel glabra, subsessilia vel fructus pedicello glandulam interdum duplo superante instructi; folia ramulorum fertilium pl.m. dense sericea vel glabra.

Flores feminei glandulis 2 (dorsali interdum minima) instructi
6c. *S. exigua* var. *nevadensis*

Flores feminei glandula tantum ventrali instructi.

Glandulae florum masculorum 2 (ventralis et dorsalis).

Ovaria etiam juvenilia glaberrima.

Folia tantum valde juvenilia pl.m. distincte sericea vel ab initio pl.m. glabra vel tenuiter pilosa pilis saepe tantum sub lente visibilibus, utrinque concoloria vel superne viridia, subtus pallidiora, saepe pl.m. glaucescentia, nervis lateralibus secundariisque pl.m. prominulis.

Amenta fructifera valde densa, fructibus condensis breviter conicis pedicello subnullo vel satis brevi glandulam vix superante instructis, bracteae florum vulgo satis obovatae et truncatae; folia subtus fere semper pl.m. pallidiora vel glaucescentia, ramulorum sterilium satis late vel elliptico-lanceolata vel oblanceolata, rarius lineari-lanceolata.

Fructus 4.5–5.5 mm. longi (pedicello brevi excluso), amenta fructifera circ. 8–9 mm. crassa; folia ramulorum fertilium 3:0.4 ad 8:1.2, interdum ad 6.5:1.5 cm. magna, citissime glabrescentia vel pilis difficile visibilibus praedita (rarius initio satis dense adpresse argyraceo-sericea), satis distanter et breviter denticulata vel pl.m. integerrima; ramuli hornotini vulgo cito glabrescentes . 7. *S. melanopsis*

Fructus ad 6.5 mm. longi, amenta fructifera ad 1.2 cm. crassa; folia ramulorum fertilium ad 9:1.5 vel 17:1.7 cm. longa vel distinctius pilosa et denticulata vel ramuli hornotini magis pilosi
7b. *S. melanopsis* var. *Bolanderiana*

Amenta fructifera satis laxiflora fructibus separatis vel ovariis fructibusque longius conico-rostratis et pedicello distincto glandulam saepe duplo superante

instructis; bracteae florum vulgo oblongiores acutioresque; folia utrinque concoloria, pl.m. linearilanceolata vel linearia vel anguste lanceolata et satis distincte subdensius denticulata.

Fructus vix ultra 6 mm. (pedicello excluso) longi; folia anguste linearia, 2–4 mm. lata, venis lateralibus vix visibilibus magis impressis quam prominulis . . . . . 6d. *S. exigua* var. *tenerrima*

Fructus (5–)7–9 mm. longi; folia interdum paullo latiora, venis lateralibus pl.m. distincte prominulis 8b. *S. longifolia* var. *pedicellata*

Folia etiam adulta pl.m. sericea, utrinque (praecipue subtus) canescentia, venis lateralibus haud vel vix prominulis, ramulorum fertilium pl.m. lineari-lanceolata, integerrima vel satis distincte remote breviter denticulata; amenta fructifera pl.m. densiflora, fructibus pedicello glandulam saepe duplo superante instructis 6b. *S. exigua* var. *stenophylla*

Ovaria semper distincte sed interdum tantum pro parte sericeo-villosa vel sericea, fructus interdum fere vel omnino glabrescentes, subsessiles vel pedicello quam glandula pl.m. breviore suffulti, rarius distincte sessiles.

Folia ramulorum fertilium pl.m. integerrima vel tantum ad apicem parce et saepe indistincte denticulata, utrinque pl.m. canescentia, satis dense sericea vel etiam adulta non distincte glabrescentia et viridia venis etiam primariis vix vel paullo prominulis; fructus satis breviter conico-rostrati, amenta fructifera densa.

Folia etiam semiadulta utrinque (praesertim subtus) dense argenteo-sericeo-villosula, ramulorum fertilium saepe satis lanceolata, vulgo ad 8–10 mm. lata; ovaria juvenilia dense et longe sericea vel sericeo-villosula; fructus ellipsoideo-conici, 5–6.5 mm. longi (confer etiam 6c. *S. exiguam* var. *luteo-sericeam*) 5. *S. argophylla*

Folia minus dense, saepe tenuiter breviter adpresse sericea, ramulorum fertilium linearia vel lineari-

lanceolata, vulgo vix ultra 8 mm. lata vel ovaria angustiora apice magis capitata (incrassata) et fructus magis elongati .....6. *S. exigua*

Folia ramulorum fertilium pl.m. distincte denticulata, vulgo cito utrinque viridescentia et glabrescentia, adulta intense laete viridia et glabra (vel in var. *Wheeleri* utrinque pl.m. sericea), nervis etiam secundariis utrinque pl.m. prominulis; fructus magis elongati et rostrati; amenta fructifera pl.m. laxiflora (si amenta sunt valde densiflora et ovaria parce vel partim pilosa conf. etiam *S. melanopsidem* var. *Bolanderianam*)...8. *S. longifolia*

Glandula florum masculorum tantum una ventralis (rarius dorsalis minima adest); amenta feminea saltem novella ovariis dense albo-sericeo-villosis subsessilibus pl.m. micantia; glandula satis lata; folia ramulorum fertilium pl.m. linearia, 4–8 cm. longa et 1–5 mm. lata, ut in *S. longifolia* dentata et nervata.....8c. *S. longifolia* var. *angustissima*

Enumeratio specierum

1. S. TAXIFOLIA Kunth in Humb. and Bonpl., Nov. Gen. Pl. **2**:18. 1817; Sargent, Silva N. Am. **9**:129. *pl. 476*. 1896; Man. Trees N. Am. 175. *fig. 147*. 1905; Sudworth, Nomencl. Arb. Fl. U.S. 123. 1897, pro parte; Britton and Shafer, N. Am. Trees 202. *fig. 164*. 1908; for further literature and synonymy see SCHNEIDER in BOT. GAZ. **65**:23. 1918.—At present I have nothing to add to what I have already stated (*l.c.*) with regard to this species and its var. *microphylla* (Schl. and Cham.) Schn. There are several forms which look rather similar to *S. taxifolia*, but differ in the shape of the anthers and some other respects. I shall discuss them under *S. exigua*.

2. S. SESSILIFOLIA Nutt. N. Am. Sylva **1**:68. 1843,[2] reprint 1852; Anders. in Öfv. K. Vet.-Akad. Förh. **15**:116. 1858; in Proc. Amer. Acad. **4**:56 (Sal. Bor.-Am. 10). 1858; in Walp., Ann. Bot. **5**:746. 1858, incl. var. *villosa;* in K. Sv. Vet.-Akad. Handl. **6**:55. *pl. 4. fig. 36* (Monogr. Salic.). 1867; in DC. Prodr. **16**[2]:214. 1868;

[2] NUTTALL'S vol. 1 was issued in 2 parts; part 1 in 1842, containing pp. 1–54; while part 2, pp. 57–136, including the Salices, appeared in 1843.

Bebb in Watson, Bot. Calif. 2:85. 1879,[3] pro parte et exclud. synon.; Sargent, Rep. For. N. Am. 10th Census U.S. 9:168. 1884, pro parte et excl. var.; Silva N. Am. 9:127. 1896, pro parte; Sudworth in Bull. U.S. Dept. Agric. Div. For. 14:122 (Nomencl. Arb. Fl.). 1897, pro parte; For. Trees Pac. Slope 223. 1908, pro parte; Eastwood, Handb. Trees Calif. 37. 1905, pro parte; Britton and Shafer, N. Am. Trees 196. 1908, pro parte minima; Howell, Fl. Northw. Am. 1:618. 1902; Piper in Contr. U.S. Nat. Herb. 11: 213 (Fl. State Wash.). 1906; Ball in Bot. Gaz. 60:49. *fig.* 2. 1915; in Piper and Beattie, Fl. Northw. Coast 115. 1915; Henry, Fl. S. Br. Col. 96. 1915; Rydberg, Fl. Rocky Mts. 192. 1917, pro parte. —*S. sessilifolia* var. *villosa* And. in K. Sv. l.c. 56 et Prodr. l.c. 214.— *S. macrostachya* Nutt., N. Am. Sylva 1:72. 1843; Howell, Fl. l.c. 619, pro parte; Rowlee in Bull. Torr. Bot. Club 27:250. 1900, pro parte et excl. var.; Rydberg, Fl. l.c. 192.—*S. macrostachya* var. *Cusickii* Rowlee, in Bull. l.c. *pl.* 9, *fig.* 5, sine descr.—*S. longifolia* var. *sessilifolia* Jones, Willow Fam. 24. 1908.

Type locality.—Oregon, "on the rocky borders of the Oregon [Columbia] at the confluence of the Wahlamet" [Willamette]. Range: from western Oregon, Douglas County, along the Umpqua and Willamette River to the Columbia and Lewis rivers in Washington, thence again in northern Washington, Whatcom County, and southwestern British Columbia.

S. sessilifolia was the only one of Nuttall's species which has been correctly interpreted by Andersson, who cites for the type *Lyall's* specimens from the Sumass Prairie, of which the male is no. 78 and the female no. 31 in Herb. K.[4] They were collected in 1858 "near the 49th parallel of lat." In the herbarium Andersson first had named the specimen *S. Grayi*, but this name has never been published. For his var. *villosa* the type was collected by *Lobb* in 1852 in Oregon, bearing the no. 218 in Herb. K. *Lobb's* and *Lyall's*

[3] Bebb's treatment of the Californian Salices in Watson's *Flora* was published separately in 1879.

[4] Besides the abbreviations mentioned in Bot. Gaz. 65:9 and 66:121, the following will be used: Cal., Herbarium of the California Academy of Science; K., Kew Herbarium; Jeps., Herbarium of Professor W. L. Jepson, Berkeley, Cal.; N.E., Herbarium of the New England Botanical Club; P., Herbarium of the Academy of Science at Philadelphia, Pa.; Reno, Herbarium of the Nevada Agric. Exper. Station, Reno, Nev.; St., Herbarium of the Leland Stanford University.

plants are exactly alike. NUTTALL himself gave an excellent description, and it is rather astonishing that the species could have ever been misunderstood. He described another species, however, *S. macrostachya*, "from the banks of the Oregon" [Columbia], of which there is a sterile(!) cotype in Herb. P. and a branchlet with an old fruiting ament in Herb. G. According to some remnants the style and the stigmas are exactly as in *S. sessilifolia*, and there is no difference in the shape and pubescence of the leaves. Judging by NUTTALL'S statement "amentis longissimis praecocibus," he had before him a very early flowering state, and the fragment in G. shows an old, almost sessile, long, fruiting ament which naturally looks very different from the normal late flowering form with the aments at the top of rather long leafy branchlets. The sheet in P. also contains a female branch of which I do not know the origin, because it is only partly represented in the photograph. It seems to me that this branchlet belongs to the true *S. argophylla* Nutt., which has a similar foliage and pubescence but shorter stigmas, looking more or less intermediate between *S. sessilifolia* and *S. exigua*. I shall deal with it later. It has been mostly taken hitherto for *S. macrostachya*. BALL (1915) also referred specimens from California to *S. sessilifolia*, but those forms I take for var. *Hindsiana*. Sterile specimens collected by *J. G. Jack* in Oregon, Josephine County, Grant's Pass, August 23, 1904, and at the same locality and time by *A. Rehder*, seem to me to belong rather to *S. argophylla* than to *S. sessilifolia*. In British Columbia, Westminster County, New Westminster, banks of Fraser River, *J. K. Henry* collected good material on June 24, 1912, and May 9 and September 25, 1914 (m., fr., st.; Cal.). The largest leaves I have seen measure up to 9:2 cm.

2b. S. SESSILIFOLIA var. HINDSIANA And. in Öfv. K. Vet.-Akad. Förh. 15:117. 1858; in Proc. Am. Acad. 4:56 (Sal. Bor.-Am. 11). 1858; in Walp., Rep. Bot. 5:746. 1858; Bebb in Watson, Bot. Calif. 2:85. 1879; Sargent, Rep. For. N. Am. 10th Census U.S. 9:169. 1884, excl. synon. var. *tenuifolia;* Eastwood, Handb. Trees Calif. 38. 1905.—*S. Hindsiana* Benth. Pl. Hartw. 335. 1857; Torrey in Pacif. R.R. Rep. 4^5:138. 1857; Newberry in Pacif.

R.R. Rep. 6^3:89. 1857; And. in K. Vet.-Acad. Handl. 6:56 (Mon. Salic.). 1867, excl. *pl. 4, fig. 37* et var.—*S. longifolia* var. *argyrophylla* f. *angustissima* And. l.c. 55; in DC. Prodr. 16^2: 214. 1868. sec. specim. Fremontii.—*S. longifolia* Greene, Man. Bot. S. Fran. Bay 299. 1894, pro parte max.—*S. sessilifolia* Sarg., Silva N. Am. 9:127. 1896, pro parte; Jepson, Fl. Calif. 339. 1909, pro parte max.; in Mem. Univ. Calif. 2:178 (Silva Calif.). 1910, prop arte max.; Ball in Bot. Gaz. 60:51. 1915, pro parte.

Type locality.—California, "ad ripas fluvii Sacramento." Range: central California to southwestern Oregon.

Of *S. Hindsiana* I have seen a photograph of the type (K.) and cotypes (G., N.) collected by *Hartweg*, which are all perfectly identical. It is closely related to typical *S. sessilifolia*, from which it differs chiefly by its more linear or narrowly lanceolate and almost always entire leaves, which are more or less distinctly petioled, and by its usually smaller and thinner aments. If it were not for some specimens which seem to combine var. *Hindsiana* with the northern *S. sessilifolia*, and others that I can hardly distinguish from the southern var. *leucodendroides* (for instance a vigorous sterile specimen from Yolo County, mouth of Buckeye Creek, lg. *R. Stinchfield*, no. 334; St.), I should take it for a distinct species. A closer study of those forms in the field is certainly needed.

There seems to occur a form with almost glabrate ovaries, judging by a specimen collected by *R. S. Ferris* in Colusa County, Sycamore Slough, April 17, 1917 (no. 619, m., f.; St.). It is otherwise rather typical var. *Hindsiana* and needs further study.

The range of this variety extends to Jackson County in southern Oregon (*Walpole*, no. 255; *Applegate*, nos. 624 and 2198) in the north, and to Monterey[5] and Kern counties in California in the south, but the southern forms like *Piper's* no. 6406 from Bakersfield come very near var. *leucodendroides*.

2c. S. sessilifolia var. leucodendroides Schneider in Bot. Gaz. 65:26. 1918.—*S. macrostachya leucodendroides* Rowlee in Bull.

[5] From this county is *Brewer's* no. 544, which came from the Nacimiento or Nacimento River or Creek, not "Narsismente" or "Nasismento" River, as the name is spelled by Rowlee and Ball according to the label in C. It is a male specimen with leaves much like var. *leucodendroides*, to which it may belong after all.

Torr. Bot. Club 27:250. *pl. 9. fig. 6.* 1900; Abrams, Fl. Los Angeles suppl. ed. 102. 1911.—*S. integrifolia* var. *leucodendroides* Rowl., l.c. sphalmate in textu.—*S. argophylla* Rowl., l.c., pro parte.—*S. exigua* var. *virens* Rowl., l.c. 256, pro parte.—*S. sessilifolia* Eastwood, Handb. Trees Calif. 37. 1905, pro parte; Britton and Shafer, N. Am. Trees 196. 1908, pro parte; Jepson in Mem. Univ. Calif. 2:178 (Silva Calif.). 1910, pro parte.—*S. macrostachya* Abrams, Fl. l.c. 101, non Nutt.—ROWLEE cites 3 specimens from southern California under his variety, namely *Parish's* nos. 2134, 2040, and 640. The last number is quoted by him also under his *S. argophylla.* It belongs to var. *leucodendroides.* No. 2134 represents an early flowering state of the male plant with small leaves and short peduncles of the catkins which measure up to 2:0.9 cm. The bracts are almost glabrate and often somewhat denticulate at apex, a fact we may also observe in other forms of *S. sessilifolia.* No. 2040, in my opinion, can be regarded as the typical var. *leucodendroides,* which seems to differ from var. *Hindsiana* chiefly in its comparatively longer and broader, very often distinctly denticulate leaves (with fine distant teeth), measuring usually from 7:1.2 to 13:1.8–2 cm. (in var. *Hindsiana* the corresponding entire leaves are about 3–10 cm. long and 3–10 mm. wide, while in the typical *sessilifolia* they measure from 5:0.8–1 to 8:3 cm., being distinctly denticulate with fine linear teeth), and by its stigmas, which usually are almost sessile and somewhat shorter and broader than in var. *typica* or var. *Hindsiana.* Some plants look almost like hybrids with *S. Parishiana* or the form of *S. exigua* from southern California. I can but repeat that a proper understanding of all these forms can only be gained by a careful study of them in the field. See also my remarks under *S. Parishiana* and *S. argophylla.*

I give an enumeration of the specimens I am inclined to refer to var. *leucodendroides,* and I should be glad to receive some information by collectors who visit these localities as to the different forms of willows growing together there.

SPECIMENS EXAMINED.—San Diego County: Santa Ysabel Creek, May 1893, *R. D. Alderson* (no. 700, f.; Cor.; ovariis parce sericeis; cited by ROWLEE under *S. exigua virens*); Mountain Spring, May 10, 1894, *E. A. Mearns* (no.

3040, m.; W.).—Riverside County: Santa Ana River, N.W. of Corona, very common, 150 m., May 26, 1918, *I. M. Johnston* (no. 1994, m., f.; A.); same place, 180 m., very common along river banks, June 9, 1917, *Crawford* and *Johnston* (no. 1244, m.; A.; St.); Temescal Canyon, along a dry wash, 400 m., May 30, 1918, *I. M. Johnston* (no. 2017, fr.; A.); same river, near Riverside, May 1888, *S. B. Parish* (no. 2040, f., type; C., Cor.); San Jacinto, along San Jacinto River, March 31, 1896, *A. J. McClatchie* (m., f.; N.; early flowering form, somewhat uncertain); eastern base of San Jacinto Mts., along the borders of the Colorado Desert, June 1901, *H. M. Hall* (no. 2105, m., f.; M.; ovariis laxe sericeis, stigmatibus mediocribus); San Jacinto River Canyon, gravelly ground along the river, common, May 12, 1918, *Durand* and *Street* (no. 23, f.; A.).—Orange County: Santa Ana River, June 1880, *S. B. Parish* (m.; A., M.; "12 ft. high").—Los Angeles County: Los Angeles, 1879, *J. C. Nevin* (m.; G.; fragment); San Gabriel River at El Monte, common along river, 90 m., May 13, 1917, *I. M. Johnston* (no. 1242, m., f.); same place, July 7, 1887, *Tracy* and *Evans* (no. 383, m.; N.); San Gabriel Mts., San Antonio Canyon, 1450 m., July 9, 1918, *F. G. Peirson* (no. 14, m.; Jeps.); canyon near San Rafael, March 31, 1888, *H. E. Hasse* (no. 3801, f.; N.; var. *Hindsianae* valde similis); sandy flat along the Los Angeles River, May 30, 1888, *H. E. Hasse* (no. 4092, m., f.; N.; stigmata iis *S. exiguae* satis similia); Los Angeles River bottom, near Los Angeles, September 9, 1917, *F. Grimmel* (fr.; St.).—San Bernardino County: San Bernardino Valley, dry sandy banks of Lyth Creek, in a large thicket, April 4, 1891, *S. B. Parish* (no. 2134, m. syntype; Cor. and C., both named *S. macrostachya* by Rowlee; "about 4 ft. high"); Lyth Creek Wash, damp land, alt. circ. 300 m., May 2, 1917, *S. B. Parish* (no. 11134, f., fr.; A.; fructibus satis glabris); vicinity of San Bernardino, alt. 300–750 m., April 8, 1899, *S. B. Parish* (no. 4591, m.; St.; 4592, f., fr.; St.; the last number represents a small-leaved form much resembling *S. taxifolia* as well as var. *Hindsiana;* needs further observation); April 13, 1903, *S. B. Parish* (no. 5197, f.; St.; same small-leaved form); May 15, 1901, *S. B. Parish* (nos. 4786, m., 4787, f., fr.; N., St.; structura florum paullo ad *S. exiguam* vergens); March 1881, *S. B.* and *W. F. Parish* (no. 640, m., fr.; A.; var. *Hindsianae* satis similis, sed stigmatibus subbrevioribus, in C. magis typica); February 20, 1881, *W. G. Wright* (nos. 10, 11, m., 12, f.; C.; "small bush 6–10 ft."); March 1 and 14, 1881, *W. G. Wright* (nos. 6, m., 7, f.; C.; early flowering specimens with short aments which look rather different); Colton, April 28, 1882, *M. E. Jones* (m., fr.; A.); Waterman Canyon, August 1900, *Shaw* and *Illingworth* (no. 4, m.; St.; amentis brevibus, antheris parvis, sed foliis normalibus); Keenbrook, Kajon Pass, May 30, 1901, *S. B. Parish* (no. 4930, f., m.; St.; very much like *S. exigua*, but the female flowers more like those of var. *leucodendroides*); same Pass, July 6, 1908, *LeRoy Abrams* and *L. E. McGregor* (no. 694, f.; St.); Cucamonga Canyon, small colony on bed of a small side canyon, alt. 900 m., May 27, 1917, *I. M. Johnston* (no.

1241,[6] m.; St.).—Ventura County: Ventura, along beach, April 17, 1916, *A. Eastwood* (no. 5034, m., 5035, f.; Cal.).—Santa Barbara County: Santa Ynez River, alt. 600 m., May 1894, *C. Franceschi* (m.; A.; quasi ad var. *Hindsianam* transiens).—Tulare County: shores of Kern River, Peppermint Valley, alt. 1440 m., July 16, 1895, *W. R. Dudley* (no. 779, m.; St.); gravelly bars of Kaweah River at Three Rivers, July 20, 1900, *W. R. Dudley* (no. 2703, st.; St.); Three Rivers, near Brittons, June 15, 1902, *W. R. Dudley* (m., fr.; St.; all these forms of Tulare County come near var. *Hindsiana;* the fruiting aments of the last specimen measure up to 6:1 cm.). See also *Brewer's* no. 544 mentioned in the preceding note.

Specimens from Kern County, Bakersfield, September 28, 1910, *E. M. McGregor* (no. 13, m.; St.), look much like *S. exigua* and need further observation. There is a specimen from Santa Barbara County, Ojai, Cliff Glen, March 15, f., April 3, 1896, m., *F. W. Hubby* (no. 56; Cor.), of which the leaves much resemble *S. taxifolia*, but those of the more vigorous shoots seem to become larger. The female flowers have 2 glands, and the stigmas are rather short but agree with those of some forms I have referred to var. *leucodendroides*. I am not quite sure about this specimen, but I strongly suspect that it is a form of var. *leucodendroides* grown in a very arid position. It is similar to *Parish's* nos. 4591, 4592 already mentioned.

3. S. FLUVIATILIS Nuttall, N. Am. Sylva 1:73. 1843; Ball in BOT. GAZ. 60:52. *fig. 3.* 1915; in Piper and Beattie, Fl. Northwest Coast 114. 1915.—*S. sessilifolia* Sargent, Silva N. Am. 9:127. *pl. 475.* 1896, pro parte, non Nuttall; Rowlee in Bull. Torr. Bot. Club. 27:250. *pl. 9. fig. 8.* 1900; Howell, Fl. Northwest. Am. 1:618. 1902, pro parte; Sudworth, For. Trees Pacif. 223. *figs. 91, 92.* 1908, pro parte; Rydberg, Fl. Rocky Mts. 192. 1917, pro parte.—NUTTALL says: "This species lines the immediate border of the Oregon [Columbia] a little below its confluence with the Wahlamet"

[6] No. 1243 of the same collector from Red Hill, near Upland, April 28, 1917, apparently represents the female form of the same willow. Mr. JOHNSTON kindly sent me the following note regarding this number: "1243 from Cucamonga Canyon. Small colonies of this willow occur in scattered localities in the lower canyons of the San Antonio Mountains; although common in the valley it is uncommon in the mountains. 1243 came from one of these isolated colonies, and from absolute knowledge I know that no other colony of this or any other LONGIFOLIAE occurs within 3 miles. The associated *Salix* spp. were *S. laevigata* and *S. lasiolepis*. Nothing like *S. exigua* occurs for miles. This is by no possibility a hybrid." Judging by the stigmas this form is more closely related to *S. exigua* than to *S. sessilifolia*. The forms of this part of S. California need a special study, and it is almost impossible to express a definite opinion on them as long as *S. Parishiana* and *S. exigua* and its varieties are not yet properly understood.

[Willamette], and "we met this species likewise on the bank of the Lewis River of the Shoshonee." The first locality has been visited by BALL, and I follow him in his interpretation of this species. Unfortunately no type specimen exists, and from NUTTALL'S statement that "the germ is smooth, with 4 sessile stigmas" I believe that he had partly *S. melanopsis* before him from the second locality quoted, which is on the Snake River in western Idaho. At present the true *S. fluviatilis* is only known from "the lower part of the Willamette River and adjacent Columbia River" in Oregon, Multnomah County, ranging eastward to Wasco County, The Dalles, where *Ball* collected it on June 24, 1915 (nos. 1997, m., 1998, 1999, f., 2000, androgyn., 2005, fr., 2007, m., 2015, fr.; C., G.). It has also been found on the opposite bank of the Columbia, in Klickitat County, Wash., by *Suksdorf*, April 23, May 31, 1881 (no. 6, f., m.; C. [7876]). Other specimens of *Ball's* (nos. 1857, 1858, 1859; fr. adult.; G.) from northeastern Utah, Cache County, Logan Canyon, above Logan, in my opinion are somewhat uncertain. They suggest certain forms of *S. melanopsis* var. *Bolanderiana*, and indeed *S. fluviatilis* seems in some respects to be quasi intermediate between *S. sessilifolia* and *S. melanopsis*. BALL himself says: "The species is quite different from the true *sessilifolia*. It is closely related to *S. melanopsis* Nutt." But he also states: "The style and stigmas indeed are very similar to those of true *S. sessilifolia*." In fact, specimens collected by BALL on the shores of the Umpqua River, near Roseburg, Oregon (no. 1961, 1962, f., fr.; G.), and distributed by him as "?*S. Bolanderiana* (×*sessilifolia*)," are somewhat similar to *S. fluviatilis*, which, however, seems to be a good species of a very local distribution, quite different in the structure of the male flowers from that of the *melanopsis* group.

4. S. PARISHIANA Rowlee in Bull. Torr. Bot. Club 27:249. *pl. 9. fig. 3.* 1900; Abrams, Fl. Los Angeles suppl. ed. 101. 1911.—*S. sessilifolia* Jepson, Fl. Calif. 339. 1909, pro parte, non Nutt.; in Mem. Univ. Calif. 2:178 (Silva Calif.) 1910, pro parte.—*S. longifolia* var. *argyrophylla* Jeps. in Mem. l.c. pro parte.—*S. argophylla* Abrams, l.c. 102, pro parte.—This is a peculiar and rather obscure species of which ROWLEE has given a somewhat unsatisfactory description.

As type is cited *Hobby's* (recte *Frank Hubby*[7]) nos. 54, 55 from Matilija Canyon, Ventura County (not in San Bernardino County or, as is written on the label of the type no. 54 before me, Santa Barbara Co.). Besides this there is given on the label for the female specimen "Cliff Glen," and for the male "Ojai Springs," localities near Matilija. The flowers are young, and the ovaries are not "densely villous" but, at least partly in no. 55, glabrescent toward the apex and base, and rather silky pubescent. The specimens could easily be taken for *S. exigua* were it not for the fact that the lobes of the stigmas are narrower, about 3 times as long as thick, and the styles distinct but short. ROWLEE also cites a specimen collected by *Coville* and *Funston* (no. 263) at Spring Valley, Inyo County, but this is sterile. Only in Herb. W. I have found a few fruits attached to it which look much like those of *S. exigua*. I find it difficult to express a definite opinion on *S. Parishiana*, but I wish to enumerate the following specimens which may represent the same form. It looks intermediate between *S. exigua* (of southern California) and *S. sessilifolia* var. *leucodendroides*, and similar forms seem to occur in the region where var. *Hindsiana* reaches the southern limit of its range. The question whether we have to do with forms of hybrid origin or with a distinct species can only be solved by careful observation in the field. See also the indications given in the key.

SPECIMENS EXAMINED.—California: Venturia County: Matilija Canyon (see the remarks given in the preceding text), April 3, 1896, *F. W. Hubby* (no. 54, m. and f. types; Cor.), April 19, 1896, *F. W. Hubby* (no. 55, fr.; Cor.); Mt. Pinos Region, Goodenough Meadow, June 28, 1896, *W. R. Dudley* and *A. F. Lamb* (no. 4717, fr.; St.; fructibus parvis vix 5 mm. longis probabiliter nondum perfecte maturis); Sespe Creek, near Ten Sycamore Flat, alt. 600–750 m., June 9, 1908, *Abrams* and *McGregor* (no. 169; G., St.); Mt. Pinos Region, below Snedden's, Lockwood Creek, June 23, 1896, *Dudley* and *Lamb* (no. 4632, st.; St.; vel *exigua*). Los Angeles County: Burbank, 1904, *J. C. Nevin* (m., fr.; St.; very near *S. exigua*); Inglewood, April 12, 1901, *LeRoy Abrams* (no. 1493, f.; St.; glandulis 2, forma incerta); Florence, old bed of the Los Angeles River, April 13, 1903, *L. Abrams* (no. 3255, m., f.; M., St.; in floribus femineis interdum glandula dorsalis adest); same county?, Leakside, *J. B. Grant* (no. 6960, f.; St.; "shrub 8 ft. high"); San Antonio

[7] For correct statements regarding this name and the following localities I am much indebted to Mr. S. B. PARISH.

Mts., Prairie, fork of San Gabriel River, moist ground in a small open flat, alt. 1700 m., August 23, 1917, *I. M. Johnston* (no. 1685 m.; St.); San Bernardino County: San Bernardino, May 15, 1913, alt. 400 m., *W. L. Jepson* (no. 5591 m., fr.; A.). Orange County: Santa Ana, spring 1902, *H. D. Geis* (no. 653 vel 553; f., fr.; St.). San Diego County: Oneonta, April 24, 1904, *H. P. Chandler* (no. 5116, f., fr., m.; N.; porro observanda); near Tia Juana, June 1895, *S. G. Stokes* (f.; St.; stigmata pl.m. sessilia, forma porro observanda); same place, April 24, 1913, *A. Eastwood* (no. 2926, m.; A.); Tia Juana River, August 1902, *A. C. Herre* (fr.; St.; ut no. 4632).—Northern Lower California: Causito(?), May 29, 1883, *C. R. Orcutt* (no. 1180, fr.; M.; ut praecedens, sed amentis duplo brevioribus, ovariis pedicello quam glandula pl.m. sublongiore instructis). Kern County: along the Santa Fe Railroad, in low moist ground about 2 miles west of Bakersfield, April 6, 1905, *A. A. Heller* (no. 7591, m., f.; A., C., M., St.; looks somewhat like *S. exigua*×var. *Hindsiana;* "shrub 6 or 8 ft. high"). Inyo County: on the old Mitchell Range, resting Spring Valley, alt. 525 m., February 6, 1891, *F. V. Coville* and *F. Funston* (no. 263, st.; W.; see preceding remarks). Tulare County: Tule River above Porterville, March 27, 1897, *W. R. Dudley* (no. 3578, f.; St.; pubescentia foliorum valde juvenilium fere ut in var. *Hindsiana*, sed ovaria parce pilosa iis *S. Parishianae* simillima).

5. S. ARGOPHYLLA Nutt. N. Am. Sylva 1:71. *pl. 20.* 1843; Rowlee in Bull. Torr. Bot. Club 27:252. 1900, pro parte; Howell, Fl. Northw. Am. 2:618. 1902, pro parte; Piper and Beattie, Fl. Palouse Reg. Wash. 53. 1901; Piper in Contr. U.S. Nat. Herb., 6:213 (Fl. Wash.). 1906, pro parte.—*S. macrostachya* Piper, l.c. 214 non Nutt.; Henry, Fl. S. Br. Col. 96. 1915.—*S. sessilifolia* Britt. and Shafer, N. Am. Trees 196. *fig. 156.* 1908, pro parte.—This species, in my opinion, has been misunderstood by almost every later author, owing probably to the inaccurate representation in NUTTALL'S plate. His Latin description runs:

Salix argophylla, foliis lineari-sublanceolatis acutis sessilibus integerrimis utrinque argenteo-sericeis, stipulis obsoletis, amentis serotinis diandris, capsulis villosis lanceolatis. Besides this he says: "This species becomes a small tree from 12 to 15 ft. in height, as silvery and white as the *Leucodendron argenteum*, the branches are brown, but the twigs are hoary with villous hairs. The leaves are very much crowded, soft, with whitish shining silky down, so abundant on either side as wholly to hide the veins, and nearly the midrib; they are also nearly without footstalks, entire on the margin, of a narrow linear outline and sharply acute, with a distinct bristly point, 1.5 to 2 inches long, and only about 3 lines wide. Stipules small and linear, seldom seen. The aments come out late with the leaves, and the flower branches produce 4–7 leaves. The male ament is small and narrow, with the scales lanceolate and villous, the female

aments are oblong, the capsules lanceolate and villous. . . . . We perceive no affinity that this species bears, except perhaps to the *S. angustifolia* of the borders of the Caspian, from which at the same time it is probably very distinct.

NUTTALL'S statements indicate that the main character of *S. argophylla* is the soft, villous, white pubescence which is also a characteristic of *S. sessilifolia* and *S. macrostachya*. He does not indicate the shape of the stigmas, owing probably to the fact that he collected only plants with mature capsules. The type locality is "one of the branches of the Oregon [Columbia], the river Boisée, toward its junction with the Shoshonee" [Snake River] in western Idaho, Canyon County. So far as I know there is no type in existence, but *Nelson* and *Macbride's* no. 1057 and *Macbride's* no. 228 from the same county seem identical with NUTTALL'S species. ANDERSSON mentioned it first in his monograph in 1867 as follows:

"*S. longifolia* ***argyrophylla:* (Nutt. Sylva Amer. p. 87 ?): foliis et capsulis tomento argenteo tomentoso-micantibus.—In regionibus meridionalibus, ut in Mexico, etc.," and he adds a forma "*angustissima:* foliis anguste linearibus." "Hab. in ripis in California (Fremont); Rocky Mountains (Nuttall)," giving as a synonym "*S. brachycarpa* Nutt. Amer. Sylva p. 85?."

In the Prodromus (1868) ANDERSSON cites under his *S. longifolia*, *argyrophylla Berlandier's* no. 2371 (recte 2341) and *Wright's* no. 1873, and adds a forma *opaca*. He certainly misunderstood NUTTALL'S species entirely, and owing to the changed spelling of the name we may regard his var. *argyrophylla* as quite a new form which has nothing at all to do with *S. argophylla*. For a further explanation of ANDERSSON'S plant see under *S. longifolia* var. *angustissima*. *S. longifolia argyrophylla* of BEBB and other authors as well as *S. fluviatilis argyrophylla* Sargent are names applied to forms of very different origin, and may sometimes include the true *S. argophylla*, but mostly seem to refer to *S. longifolia* var. *Wheeleri*. ROWLEE (1900) mixed with it *S. Hindsiana* Benth. and also forms which belong to *S. exigua* and *S. sessilifolia leucodendroides*. PIPER (1906) and BALL (in different herbaria) referred the forms I take for *S. argophylla* mostly to *S. macrostachya*, but NUTTALL'S type of this species belongs to *S. sessilifolia*, as previously explained.

Male or sterile specimens of *S. argophylla* are not always easily separated from *S. sessilifolia*, as for instance those collected by

Jack and also by *Rehder* on Grant's Pass, Oregon. The female plants show almost the same stigmas as in *S. exigua*, and *S. argophylla* looks often quite intermediate between this species and *S. sessilifolia*.

So far as can be judged at present by the specimens enumerated, its range seems to extend from Bonneville County in eastern Idaho, along Snake River to Canyon, Washington, and Nez Perces counties, and into adjacent Washington (Walla Walla, Whitman, and probably also Franklin and Lincoln counties) as far as western Klickitat County, while in Oregon the species occurs in Sherman and Wasco counties, the forms from Klamath and Josephine counties being rather uncertain. The male specimen from British Columbia cited later looks much like *S. sessilifolia*, but Professor PIPER, with whom I have had an opportunity to discuss the matter, believes it is better referred to *S. argophylla* for geographical reasons. Only a close study in the field, especially of the forms of southern Washington and northern Oregon in the region of the Columbia and its tributaries, can elucidate the relationship of *S. argophylla* with *S. sessilifolia* and the limits of their geographical distribution. At present I can hardly do more than to indicate what form has to be taken for NUTTALL's *S. argophylla*, and how it seems to be related to and connected with either *S. sessilifolia* or *S. exigua*. It would be rather misleading to make too decisive statements as long as one's information is merely based on herbarium material.

SPECIMENS EXAMINED.—Idaho: Bonneville County: Idaho Falls, among rocks, along river, July 4, 1901, *E. D. Merrill* and *E. N. Wilcox* (no. 803, m.; G.; "4–5 ft."); Canyon County: Falk's Store, slough and creek banks, alt. 660 m., July 11, 1911, *A. Nelson* and *Macbride* (no. 1057, fr.; G., M., St.); along the river, same alt., June 7, 1910, *J. F. Macbride* (no. 228, m.; G., M., St.); Caldwell, irrigation ditch, October 1, 1910, *C. R. Ball* (no. 1705, fr.; W.; "10 ft."); Washington County: Weiser, alt. 660 m., July 5, 1899, *M. E. Jones* (no. 6554, f., fr.; W.); ?Nez Perce County: Clear Water River, June 18, 1894, *L. F. Henderson* (f., fr., non m.; W.; same as no. 2878 in C.; forma satis ad *S. exiguam* spectans).—Washington: Walla Walla County: Waitsburg, June 24, 1897, *R. M. Horner* (R. 454, B. 451, m.; G., W.); Whitman County: Wawawai, July 9, 1901, *C. V. Piper* (no. 3592, m.); same place and collector, June 13, 1901 (no. 3595, m.); West Klickitat County: Columbia River, damp or wet places, May 31, July 1884, *W. N. Suksdorf* (m., f., fr.; C.,

M., St.); Franklin County: Pasco, June 1902, *H. P. Baker* (no. 70, m.; M.; vel ad *S. sessilifoliam* referenda); Lincoln County: Sprague, alt. 560 m., June 3, 1893, *J. H. Sandberg* and *J. B. Leiberg* (no. 134; W.; forma porro observanda, paullo ad *S. exiguam* spectans).—Oregon: Sherman County: Biggs, along stream, 1 mile south of Columbia River, August 1, 1914, *C. R. Ball* (no. 1848, fr.; W.); Wasco County: Tygh Valley, June 1881 (vel 1880), *T. J. Howell* (m. vel androgyn.; A., M.); Hood River County, Hood River, May 25, 1879, *J. T. J. Howell* (m. vel androgyn.; C.); Klamath County: along Sprague River above Yainax Valley, *F. V. Coville*, August 23, 1902 (no. 1312, st.; forma quamvis incerta); Josephine County: Grant's Pass, August 23, 1904, *J. G. Jack* (st., A.; forma incerta); same place and date, *A. Rehder* (st.; "large shrub"; ut praecedens); ? County: Cache Bar, between Cache and Gordon creeks on Snake River, alt. 380 m., June 19, 1897, *E. P. Sheldon* (no. 8325, m.); east Oregon, without exact locality, stream banks, May 9, June 7, September 1898, *W. C. Cusick* (no. 1860, m., f., fr.; M.; "a straight upright shrub"; forma foliis lanceolatis satis denticulatis).—British Columbia: Kootenay District, Cascade, near international boundary between Kettle and Columbia rivers, June 26, 1902, *J. M. Macoun* (no. 68128, O.; m.; G.).

6. S. EXIGUA Nutt. Sylva N. Am. **1**:75. 1843; Rowlee in Bull. Torr. Bot. Club **27**:255. *pl. 9, fig. 15.* 1900, pro parte; Piper and Beattie, Fl. Palouse Reg. Wash. 53. 1901; Howell, Fl. Northw. Am. **1**:618. 1902; Piper in Contr. U.S. Nat. Herb. **6**:213 (Fl. Wash.). 1906; Britton and Shafer, N. Am. Trees 195, *fig. 155.* 1908; Ball in Coult. and Nels., New Man. Rocky Mts. Bot. 131. 1909; Garrett, Spring Fl. Wasatch Reg. 10. 1901, pro parte; ed. 2. 16. 1912, pro parte; Rydberg, Fl. Rocky Mts. 192. 1917, pro parte.—*S. longifolia* var. *β* Hooker, Fl. Bor. Am. **2**:149. 1839, quoad specim. Tolmieana.—*S. longifolia* Wats., Cat. Pl. Nev. Utah, in King's Rep. **5**:324. 1871, quoad specim. no. 1094, non Muhl.; Bebb in Coult., Man. Rocky Mts. Bot. 335. 1885, pro parte; Jeps. in Mem. Univ. Calif. **2**:178 (Silva Calif.) 1910, pro parte.—*S. longifolia* var. *exigua* Bebb in Wats., Bot. Calif. **2**:85. 1879; Jones, Willow Fam. 24. 1908, pro parte.—*S. longifolia* var. *argyrophylla* Macoun Cat. Can. Pl. **1**:450. 1883, pro parte; Jeps. in Mem. l.c. pro parte.—*S. fluviatilis* var. *exigua* Sarg., Silva N. Am. **9**:124. 1896, pro parte; Sudworth in Bull. U.S. Dept. Agr. Div. For. **14**:122 (Nomencl. Arb. Fl.). 1897, pro parte max; For. Trees Pacif. Slope 223. 1908.—*S. longifolia* var. *argophylla* Jones, Willow Fam. 24. 1908, pro parte.—*S. argophylla* Henry, Fl. S. Br. Col. 96. 1915;

Rydbg., Fl. Rocky Mts. 188. 1917.—The type of this species was collected by *Nuttall* with his *fluviatilis*, probably "on the banks of the Lewis River of the Shoshonee" (Snake River in Idaho), because at the type locality of *S. fluviatilis* on the Columbia in the vicinity of Portland, Oregon, this species is apparently the only one of the LONGIFOLIAE according to BALL (BOT. GAZ. 60:45, in note, 1915). *Nuttall* says: "This species is also a native of the territory of Oregon, and grew with the preceding, which it strongly resembles" (*S. fluviatilis*); he does not indicate the exact locality. I have a photograph of a so-called cotype of *S. exigua* from Herb. P. consisting of a sterile branchlet. The label originally bore the inscription "*S. longifolia*, Missouri and Arkansas." The name *longifolia* has been crossed out, and in a similar handwriting is written "*exigua* Nutt." Judging by the serration and nervation of the leaves there can be no doubt that the specimen belongs to *S. longifolia*. I do not know of a true type specimen of *S. exigua*, but there can hardly be any doubt as to the form NUTTALL had in mind. From his phrase "capsulis lanceolatis sessilibus, demum nudiusculis" I infer that the typical *S. exigua* is a form with, at least in the beginning, hairy ovaries, but ROWLEE and other authors ascribe to it glabrous capsules. BALL (1909) is right in stating that it is "variable in foliage characters and sometimes very difficult to distinguish" from *S. longifolia*. In spite of having seen an abundant and well collected material, I am still at a loss how to define certain forms and to draw a sharp line between *S. exigua* on the one hand and such species as *S. longifolia*, *S. argophylla*, *S. Parishiana*, and also *S. taxifolia typica* on the other. From *S. longifolia* and its forms it differs chiefly in the opaque color of the canescent leaf-surfaces, bearing a more or less dense appressed tomentum of short silky hairs (especially on the young leaves) of a silvery hue. The leaves are usually smoother with a hardly visible nervation, but in old leaves (for instance in those of the southern form) the veins are sometimes rather well marked; their margin is mostly entire, but a dentation similar to that of *S. longifolia* may be observed in the southern forms. The fruiting aments usually are denser and the capsules as a whole shorter. *S. argophylla* chiefly differs, as previously stated, by its more villous tomentum, while *S. Parishiana*,

which cannot be distinguished by its pubescence, may be recognized by the longer lobes of the stigmas and the more or less distinct style. Male specimens of these species sometimes prove difficult to distinguish. In *S. sessilifolia leucodendroides* the base of the leaves usually is more obtuse and suddenly contracted in the very short petiole, while in *S. exigua* as well as in *S. Parishiana* the leaves are mostly attenuated at the base, passing gradually into the somewhat longer petioles. *S. Parishiana* normally has linear leaves, while in *S. exigua* they are more linear-lanceolate, but all those characters have to be taken cum grano salis. There is a specimen before me from southern New Mexico, Dona Ana County, Mesilla, alt. 1150 m., June 19, 1897, *E. O. Wooton* (no. 39, m.; G., St., W.), of which the younger leaves are almost sessile, with a pubescence like those of var. *leucodendroides*, but are more linear; the older ones, which are more glabrescent and measure up to 12 by 0.5 cm., have a distinct petiole 2–3 mm. long. The pubescence and shape of the bracts seem to vary in the same manner in every species. Whether or not the shape and size of the anthers afford a useful character I cannot state. In those regions where the species meet each other hybrid forms are certain to occur.

The range of what I call the typical form of *S. exigua* extends from southern Idaho (from which the type probably came) westward to Oregon (where the western line seems to run from about Wasco County to Klamath County) and Washington (where it hardly reaches the eastern slopes of the Cascades), northward to British Columbia (where I did not see it from farther north and west than Clinton on the Fraser River) and southern Alberta (Medicine Hat), eastward to central Montana and western Wyoming (Yellowstone Park), and southward to southeastern Nevada and southern California. In California it seems to occur along the eastern border line from Modoc to Inyo County (Panamint Range), and in the south (Ventura to San Bernardino, Imperial, and San Diego counties). There are also forms very near to it in San Benito, Tulare, and Kern counties, which partly point toward *S. sessilifolia* var. *Hindsiana*. From the south I also have seen forms which come very near var. *leucodendroides* on the one hand and *S. Parishiana* on the other. As already stated, the limitation of these species is a very difficult task.

In Nevada and Utah a form is found in which the female flowers have a ventral and a dorsal gland. To this form belongs *S. nevadensis* Wats., the type of which came from Nevada, Ormsby County, near Carson City. It is certainly not a good species, but I am inclined to keep it as a variety until it is proved by further observation that the presence of a dorsal gland is a character of no taxonomic value, and that no other character can be detected by studying the plant in the field. In proposing the name S. EXIGUA var. **nevadensis,** nov. var. (*S. nevadensis* Watson in Am. Nat. 7:302. 1873), I provisionally refer to it the following specimens, and wish to draw the attention of collectors to the localities mentioned. The type has glabrous ovaries, with a pedicel nearly as long as the ventral gland, while other forms with two glands have a more or less dense pubescence.

SPECIMENS EXAMINED.—Nevada: Ormsby County, at the base of the Washoe Mountains, near Carson City, alt. 1500 m., April 1868, *S. Watson* (no. 1093, f. type; G.); same region, 1865, *C. L. Anderson* (no. 196, m., fr.; G.; ovaria pilosa); Washoe County, Franktown Creek, May 18, 1907, *C. L. Brown* (no. 1677, f.; Reno); Glendale, alt. 1300 m., May 1, 1909, *P. B. Kennedy* (no. 1743, m.; G.); sloughs between Pyramid and Winnemucca lakes, alt. 1250 m., June 2, 1913, *P. B. Kennedy* (no. 1996, m., fr.; G.; forma quasi ad *S. sessilifoliam* var. *Hindsianam* accedens); Truckee River, alt. 1350 m., June 6, 1913, *P. B. Kennedy* (no. 2010, m., f.; G.); central Nevada, without exact locality, 1871, *Wheeler* (m.; syntype; G.).—California: Nevada County, along Coldstream, 3 miles above Truckee, July 17, 1913, *A. A. Heller* (no. 6953, fr.; forma aliquid incerta).—Utah: Washington County, St. George, alt. 600 m., April 9, 1880, *M. E. Jones* (no. 1644, m., f.; A., C.); without date, *E. Palmer* (no. 8, m., f.; M.); Redsand, alt. 900 m., April 24, 1894, *M. E. Jones* (no. 5117, m., f.; M.); Santa Clara, 1874, *C. C. Parry* (no. 8, m., f.; M.); Beaver County, Milford, along a stream, June 4, 1902, *L. N. Goodding* (no. 1018, fr.; W.); plains and mountains east of Milford, June 22, 1905, *P. A. Rydberg* and *E. C. Carlton* (no. 6318, fr.; G.); Salt Lake County, Salt Lake City, 1350 m., May 1869, *S. Watson* (no. 1091, fr.; G.); same place, May 12, 1880, *M. E. Jones* (no. 1710, m., f.; A., C.); Davis County, Lagoon, common, alt. 1500 m., July 7–8, 1901, *Pammel, Johnson, Buchanan,* and *Lummis* (fr. adult.; M.; probably var. *typica*).—Idaho: Bear Lake County, Montpelier, creek banks, May 20, 1910, *J. F. Macbride* (no. 207, f.; G.); Power County, north of Arbon, bridge over Bannock River, August 6, 1915, *C. R. Ball* (no. 2020, st.; G.; forma incerta).

There are also the following 2 specimens from southern California which resemble *S. exigua* and possess 2 glands in the female flowers: San Bernardino

County, Cushenberry[8] Spr[ing], Mojave Desert, June 2, 1901, *S. B. Parish* (no. 4931; N., St.), and Los Angeles County, Los Angeles, April 1901, *G. B. Grant* (no. 1156; M.; apparently the same as *Parish's* plant). Both need further observation.

In 1900 ROWLEE described a *S. exigua* var. *virens* (Bull. Torr. Bot. Club **27**:255), for the type of which a specimen collected by *Rothrock* in Arizona has to be taken. So far as can be discovered from the specimens cited by the author, I believe that ROWLEE mixed several forms of different affinity, belonging partly to *S. melanopsis Bolanderiana* (*Bolander*, no. 5031; *Kellogg* and *Harford*, no. 922; *W. G. Wright*, Kernville [not Kernerville]), and partly to *S. sessilifolia leucodendroides* (*Alderson* [not *Anderson*] no. 700). The type of *Rothrock*, which is sheet no. 6122 in C., represents a female specimen of which the flowers can hardly be distinguished from those of *S. exigua*. In the leaves it agrees well with a male specimen of *Orcutt's* (San Diego County, in the southwestern part of the Colorado Desert, Dos Cabesas, October 11, 1890, no. 2227; A., C.), which number is also cited by ROWLEE. Both may be taken for a rather glabrescent variety of *S. exigua*, but the leaves show under the lens a fine and thin silky pubescence and cannot be called "nearly glabrous," a character apparently taken by ROWLEE from the specimens of var. *Bolanderiana*. *Rothrock's* and *Orcutt's* specimens come very near the 2 specimens of *Parish* and *Grant* with 2 glands in the female flowers. Besides these there is *Parish's* no. 3194 (San Bernardino County, San Bernardino Mountains, Big Morongo, alt. 900 m., June 15, 1894; m.; M.) that hardly differs from *Orcutt's* plant, and also *LeRoy Abrams'* and *McGregor's* no. 406 (Los Angeles County, Liebre Mountains, Oakgrove Canyon and Elizabeth Lake, June 20–23, 1908; f., fr.; St.) seems to represent such a form the leaves of which become rather greenish at maturity, but the lower surface is rather glabrescent in *Rothrock's* specimens. This form somewhat simulates var. *Bolanderiana*, and I cannot express at present a definite opinion as to its real taxonomic value and true affinity.

[8] ROWLEE spells the name Cashewberry, but I read it as given, and S. B. PARISH writes in a letter to Professor C. S. SARGENT that this is the local way of spelling the name, while on the map of the Geological Survey it is spelled Cushenbury.

In Bot. Gaz. 65:25. 1918 I have made *S. stenophylla* Rydbg. a variety of *S. exigua*, referring to it the eastern and southeastern forms of this species. Rydberg's female type and male syntype came from southern Colorado, Huerfano County, Cuchara River, below La Veta (*Rydberg* and *Vreeland*, nos. 6393 f., 6392 m.; N.), and the ovaries are only partly glabrous, while most of the forms I take for var. *stenophylla* have wholly glabrous ovaries and fruits. The main character by which they differ from typical *S. exigua* is the longer pedicel, which in the fruit usually surpasses the gland in length. After all, even this character can scarcely be regarded as constant, and var. *stenophylla* is connected with the typical form by numerous intermediates. As a whole, however, the forms of *S. exigua* from Wyoming, Colorado, Arizona, New Mexico, Texas (Randall and El Paso counties), and probably also on the western border of Kansas, in northwestern Oklahoma, and in northern Mexico (northern Chihuahua), seem to present slight variations and may be called var. *stenophylla* until further studies in the field have led to a more proper understanding of the variability of this species. I suggested in Bot. Gaz. 65:25. 1918 that *S. Hindsiana* var. *tenuifolia* And. (in K. Sv. Vet.-Akad. Handl. 6:56. 1867) might be identical with var. *stenophylla*, in which case the name *tenuifolia* would have to be used. As type a specimen collected by *Burke* on the banks of the Snake River near Fort Hall in Idaho has to be taken. Judging by a photograph and fragments of the type preserved in Herb. K. I cannot decide whether the male specimen really belongs to what I call var. *stenophylla* or to the typical *S. exigua*. It comes from a region where both forms meet. The second specimen cited by Andersson "Nova Mexico (*Schur*)" is unknown to me, and may probably be referable to var. *stenophylla*, which name I prefer to keep so long as the identity of the Snake River form remains uncertain. To var. *stenophylla* also partly belongs as a synonym *S. longifolia* * * * *opaca* And. (in K. Sv. Vet.-Akad. Handl. 6:55. 1867) in so far as it refers to *Wright's* no. 1873, while *Berlandier's* no. 2341 represents *S. longifolia angustissima*.

In western Nebraska and northeastern Colorado another form of *S. exigua* has been found which somewhat reminds one of the f.

Wheeleri of *S. longifolia* (see following). RYDBERG described this form as *S. luteosericea* (in Britton, Man. 316. 1901) and kept the name in his Fl. Color. 94. 1906, while he makes it a synonym of his *S. exigua* in 1917 (Fl. Rocky Mts. 192), as BALL has already done in 1909. The type came from western Nebraska, Banner County. I think it best at present to keep this form separate under the name S. EXIGUA var. **luteosericea,** nov. var., and I provisionally refer the following specimens to it in the hope that collectors may pay attention to the localities mentioned and try to get a better understanding of this variety by studying it carefully in the field as to its association with typical *exigua* and with *S. longifolia*. I can hardly point out a good character by which to recognize this form, but its pubescence is a little more villose, and the aments are more loosely flowered than in typical *exigua* or var. *stenophylla*.

SPECIMENS EXAMINED.—Western Nebraska: Banner County, Lawrence Fork, July 8, 1891, *P. A. Rydberg* (no. 368 partim, f. type; N.); Kearney County, dry creek, June 13, 1891, *P. A. Rydberg* (no. 369, m. syntype; N.); Scotts Bluff County, Platte bottom, in Mitchell Valley, August 4, 1891, *P. A. Rydberg* (no. 368 partim, fr.; N.).—Colorado: Weld County, Greeley, July 23, 1896, *L. H. Pammel* (no. 200, fr., 201, m.; M.); Larimer County, without exact locality, plains, alt. 1500 m., June 26, 1895, *C. F. Baker* (*Patterson* no. 9842, m., f., rather typical, the male specimen almost identical with *exigua typica*); Fort Collins, near river, June 26, 1896, *L. H. Pammel* (no. 202, f.; M.); same locality, meadow near river, August 6, 1898 (Hb. Agr. Coll. Colo., no. 2343, fr.; C.); Morgan County, Fort Morgan, June 1896, *L. H. Pammel* (no. 204, st.; M.); Fremont County, Canyon City, banks of the Arkansas River, September 24, 1874, *G. Engelmann* (st., M.; vel var. *stenophylla*); Boulder County, August 1 [and 21 ?], 1884, July 20, 1885, *G. W. Letterman* (fr.; M.); Denver County, Denver, August 20, 1884, *G. W. Letterman* (fr.; M.).—S. Dakota: Butte County, Indian Creek, along flood plain, July 31, 1911, *S. S. Visher* (no. 2640, st.; C.; f. incerta); Bennett County, Little White River, valleys, August 15, 1911, *S. S. Visher* (no. 2274, st.; C.; rather uncertain, similar to *S. longifolia Wheeleri*).

There remains another form the proper interpretation of which raises many difficulties. It was described by HENDERSON as *S. longifolia tenerrima* from specimens collected by the author in Idaho, Elmore, and Canyon counties. At first sight it can hardly be distinguished from what I call *S. longifolia* var. *pedicellata* (see later), especially from such specimens as *Eastwood's* no. 465, but a

closer inspection shows that the leaves as a whole are narrower and the fruits shorter. In this respect it agrees more with *S. exigua*, of which it would represent an extremely glabrescent form. HELLER made it a species, and BALL evidently took the same view, as shown by his determinations of herbarium specimens before me, but RYDBERG (1917) quotes it as a synonym of his *S. linearifolia*, which is the same as var. *pedicellata* of *longifolia*. So far as I know the range of this peculiar form, it seems to be restricted to southwestern Idaho (region of Boise River), northwestern Wyoming (Yellowstone Park and northern Lincoln County), and adjacent southern Montana (Carbon and Big Horn counties). There is, to my present knowledge, no *S. longifolia* in this region, but it is within the range of *S. exigua*. I am therefore inclined to follow a suggestion of C. V. PIPER, with whom I have discussed this question, and to refer var. *tenerrima* as a variety to *S. exigua*.

S. EXIGUA var. **tenerrima,** nov. comb.—*S. longifolia* var. *tenerrima* Henderson in Bull. Torr. Bot. Club 27:354. 1900.—*S. tenerrima* Heller, Cat. N. Am. Pl. ed. 2. 4. 1900.—*S. fluviatilis* var. *tenerrima* Howell, Fl. N.W. Am. 618. 1902.—*S. linearifolia* Rydbg., Fl. Colo. 94. 1906, ex parte; Fl. Rocky Mts. 192. 1917 ex parte.—A typo praecipue differt foliis angustioribus linearibus etiam maximis vix ultra 4 mm. latis juvenilibus ut rami novelli parce breviter sericeis cito glabris vel pilis parcis difficile recognoscentibus vestitis utrinque satis viridibus vix nervatis vulgo pl.m. distincte denticulatis dentibus brevibus subglandulosis saepe satis distantibus, ovariis subsessilibus glabris, bracteis oblanceolatis tantum versus basim pilosis, fructibus vulgo pedicello distincto glandulam duplo superante instructis conico-rostratis pedicello excluso ad 6 mm. longis.

SPECIMENS EXAMINED.—Idaho: Elmore County, shady rocky banks of mountain rills gone dry, July 12, 1895, *L. F. Henderson* (fr., type; G.); Canyon County, Payette River, sandy bottoms, August 1, 1897, *L. F. Henderson* (fr.; G.); Falk's Store, open sandy slopes, alt. 660 m., May 24, 1910, *J. F. Macbride* (no. 98 m., fr. juv.; G., M., St.; "loose clumps").—Wyoming: Yellowstone Park, Soda Butte Creek, July 14, 1899, in small clumps on the stony river bottom, *A.* and *E. Nelson* (no. 5866, fr.; G., St.); Lincoln County, Jackson's Hole, banks of Gros Ventre River, July 14, 1901, *S. D. Merrill* and *E. N. Wilcox* (no. 996, fr.; G., M.; "10 ft.").—Montana: Big Horn County, Crow Agency,

August 30, 1871, *Coulter* (no. 5, st.; C.; forma porro observanda); Carbon County, near Red Lodge, July 28, 1893, *J. N. Rose* (no. 50, fr. adult.; forma aliquid incerta); Gallatin County, Bozeman, Gallatin River, low ground, October 4, 1905, *J. W. Blankinship* (no. 465, st.; A.; forma incerta ad *S. longifoliam pedicellatam* spectans); Rosebud County, Forsyth, north of town, toward river, 1908, *C. R. Ball* (no. 1305, st.; G.; "6 ft. high"; forma porro observanda).—Utah: Cache County, Logan Canyon, above Logan, August 8, 1914, *C. R. Ball* (no. 1864, fr.; W.; forma glabra pro *S. exigua* determinata, porro observanda).

This variety needs further observation in the field, and some of the specimens cited are uncertain owing to the lack of fertile material. Some forms of *S. longifolia pedicellata* are extremely alike, but the leaves show a more or less prominent (often very fine) venation, while in the leaves of var. *tenerrima* the lateral veinlets are scarcely visible and finely impressed; the fruits of both are sometimes almost identical, and I am not yet sure of the true affinity of var. *tenerrima*. *G. J. Jack*, August 16, 1918, collected on the Laramie River, Laramie, Albany County, Wyoming (no. 1017), sterile specimens of a form of which I am not sure whether it is var. *tenerrima* or var. *pedicellata*, neither of which has hitherto been reported from southeastern Wyoming. Professor JACK says: "Slender, coarse, grasslike, 2–3 ft. high, covering wide sandy areas," and he told me that it is a very distinct low form. There are now living plants in the Arnold Arboretum which I hope will prove useful in determining its real affinity.

There is still one form which needs a few words. It was collected by *S. M. Tracy* and *F. S. Earle* in western Texas, Jeff Davis County, Limpia Canyon, April 24, 1902 (no. 210, fr.; C., G.; distributed as "*S. longifolia opaca* Ands."), and it seems to be identical with Mexican specimens mentioned by me in BOT. GAZ. 65:23. 1918, under *S. taxifolia*. The habit and the leaves agree well with those of that species, but the fruits in no. 210 are much more like those of *S. exigua* with short sessile stigmas. It looks almost like a new species closely related to *S. exigua*, which seems to show a variability remarkable even among willows.

7. S. MELANOPSIS Nuttall, N. Am. Sylva 78. *pl. 21*. 1843; Rowlee in Bull. Torr. Bot. Club 27:256. *pl. 9, fig. 16*. 1900, pro parte; Piper and Beattie, Fl. Palouse Reg. Wash. 53. 1901; Piper in Contr. U.S.N. Herb. 11:213 (Fl. Wash.). 1906, pro parte; Ball

in Coult. and Nels., New Man. R. Mt. Bot. 131. 1909; in Piper and Beattie, Fl. Northw. Coast 114. 1915; Henry, Fl. S. Br. Col. 97. 1915; Rydberg, Fl. R. Mts. 192. 1917.—*S. longifolia* Bebb apud Coulter, Man. R. Mt. Bot. 335. 1885, pro parte, non Muhl.—*S. fluviatilis* Howell, Fl. Northw. Am. 1:618. 1902, pro parte, non Nutt.—This is a well marked species the type of which was found by NUTTALL "at our station called Fort Hall, in the plains of the Rocky Mountains, on alluvial lands of Lewis River of the Shoshonee." According to BALL (1909), this is old Fort Hall, near Pocatello, in Bannock County, eastern Idaho, south of the present Fort Hall,[9] near Blackfoot, in Bingham County. I have seen a photograph of a cotype preserved in Herb. P. BALL (1909) gives the range as follows: "Common in northeastern Oregon, eastern Washington, and British Columbia as far east as the Selkirks." I have not seen a specimen from the type region or other parts of southern Idaho, but only from northern Idaho, Montana (Teton County, Midvale, *L. M. Umbach*, no. 170), Alberta (Crow Nest Pass and Jasper), where it seems to reach its northern limit at about the 53d parallel, British Columbia (in the Chilliwack Valley and at Revelstoke), Washington (where I have seen it west of the Cascades only from King County, Snoqualmie), Oregon (where it was collected by *Ball* in 1915 as far west as the Umpqua River, Roseburg, Douglas County, and by *Applegate*, no. 2224, at Ashland, Jackson County), and northern and northeastern California (see below), where it seems to pass into var. *Bolanderiana*. According to BALL (BOT. GAZ. 60:45, first note, 1915), *S. Bolanderiana* is associated with *S. sessilifolia* at Roseburg and also farther north "on the Willamette River at Corvallis," Benton County. What I have seen from Oregon I take for the true *S. melanopsis*, which ought to be looked for also in northern Utah and in western Wyoming.[10] Its

[9] This locality, however, is identical with that given for Fort Hall in Lippincott's Geogr. Dict., ed. of 1855; while on the map in the Century Atlas of 1911 old Fort Hall is indicated south of the 43d parallel just north of Pocatello. Judging by Rand McNally's map the whole region between the two places is called Fort Hall.

[10] There is a specimen from eastern Wyoming, Converse County, Rawhide Creek, south of Patrick, August 27, 1901, *H. P. Baker* (m.; M.), which looks like typical *S. melanopsis*. In Herb. C. I found a specimen from Colorado, Clear Creek County, damp places along Clear Creek, 1885, *H. N. Patterson* (fr. adult. [sheets 5523 and 107801]), which clearly resembles *S. melanopsis*. I am not sure whether the localities given are correct.

occurrence so far north in Alberta is interesting. In the north a form with more hairy, almost shining silky leaves seems to be not infrequent (see *J. Macoun's* specimen from Lower Arrow Lake, no. 24569, O.). The species has usually been mistaken for *S. longifolia* or *S. fluviatilis*, but apparently it forms with the southern var. *Bolanderiana* a well marked type in this section, and I am not yet sure to which other group of it *S. melanopsis* is most closely related. BALL (BOT. GAZ. **60**:51. 1915) speaks of a "*S. fluviatilis-melanopsis*" aggregation in contrast with the *S. sessilifolia* group, but I think *S. melanopsis* has very little to do with the true *S. fluviatilis*. The specimens from Umatilla County, Oregon, western slope of the Blue Mountains, in a swampy meadow at Ukiah, June 24, 1908, *W. Cusick* (nos. 3260, 3261, fr. juv.; N., St.), need further observation. The young fruits show a short style and are almost sessile. The main characters of *S. melanopsis* may be gathered from the key. The species is not even mentioned by ANDERSSON (1858, 1867, 1868), and its identity has first been revealed by ROWLEE (1900), who erroneously states that "it is particularly abundant along the Columbia River where NUTTALL saw it." I have not seen all the specimens cited by ROWLEE, but those of *Coville*, from Washington, Cowlitz County, north fork of Lewis River, July 16, 1898 (no. 719, fr.; W.), which are not mentioned in PIPER'S *Flora* and which have leaves that measure up to 9:2.2 cm., seem not to represent typical *S. melanopsis*, and I have not yet been able to identify them properly. In Herb. C. are similar specimens collected by *W. N. Suksdorf* in W. Klickitat County, "rocky bank of the Larm River," July 17, 1884. After all they may be taken for a form of *S. melanopsis* with very broad leaves. In California *S. melanopsis* is mostly represented by the following variety:

7b. S. MELANOPSIS var. **Bolanderiana,** nov. var.—*S. longifolia* Bebb in Watson, Bot. Calif. **2**:84. 1879, pro parte, non Muhl.; Jepson, Fl. Calif. **2**:340. 1909, pro parte; in Mem. Univ. Calif. **2**:178 (Silva Calif.). 1910, pro parte.—*S. Bolanderiana* Rowlee in Bull. Torr. Bot. Club **27**:257, *pl. 9, fig. 12.* 1900.—*S. exigua* var. *virens* Rowlee, l.c. 255, *pl. 9, fig. 11.*—*S. argophylla* Rowlee, l.c. 252, quoad specim. *Bolanderii* (non *Breweri!*) no. 5031.—*S. fluviatilis* Eastwood, Handb. Trees Calif. 37. 1905, pro parte, non Nutt.; Sudw.,

For. Trees Calif. Slope 222. *fig. 91.* 1908, pro parte.—Of this variety ROWLEE has given a very incomplete description, and in citing the specimens he says "*Bolander*, nos. 49, 58, 4958, 5031." There are no nos. 49 and 58 of *Bolander*, but only no. 4958, which has to be taken for the type. No. 5031 is also cited by ROWLEE under *S. exigua* var. *virens*, of which I previously have spoken, and again under *S. argophylla* as a number of *Brewer*, who, so far as I know, never collected a specimen bearing the same number at the same locality from which *Bolander's* plant came.

This variety differs from the type chiefly by the characters indicated in the key. ROWLEE's statement in his key that in *S. melanopsis* the leaves are "distinctly glaucous and prominently veiny beneath" while they are "not distinctly glaucous nor veiny beneath" in *S. Bolanderiana* is not correct. The leaves are sometimes rather greenish beneath in both forms. The typical form of var. *Bolanderiana* is somewhat pubescent, while most of the specimens before me belong to a glabrous form. There can also be observed a slight variation with partly hairy ovaries and fruits in the specimens of *J. Burtt Davy* (no. 5691, from Hoopa Valley, Humboldt County, California) and *S. Watson* (no. 1092, Truckee Valley, Washoe County, Nevada). Both need further observation, and may represent hybrids with *S. exigua*. This seems also the case with *A. A. Heller's* no. 6953 (along Coldstream, 3 miles above Truckee, July 17, 1908). On the other hand, specimens collected at Sunol Valley, Alameda County, June 29, 1916, by *L. R. Abrams* (no. 5692, no. 5693, f.; St.), of which the male plant cannot be distinguished from typical var. *Bolanderiana*, possess ovaries and fruits which are hairy throughout or become glabrous only to a slight degree. They do not look like hybrids, and seem to represent a distinct form with pubescent ovaries and rather silky tomentose young leaves.

The typical *S. Bolanderiana* has rather broad leaves, but there are before me many very narrow leaved specimens, and further observation in the field must show whether the forms with linear-lanceolate leaves can be separated from the typical form. I do not wish to propose too many new varieties and forms which are only known to me from herbarium specimens, but I believe that a

closer study of many difficult forms which I can only briefly mention will lead to a different conception of them.

I have seen specimens of var. *Bolanderiana* from the following counties in California (north to south): Humboldt, Siskiyou (*A. A. Heller*, no. 8058, female part not quite typical), Shasta, Lassen, Plumas, Butte, Nevada, ?Mendocino (*A. Kellogg* and *W. G. W. Hartford*, no. 922, ?Ukiah), Lake, Solano, Alameda (Sunol), Amador, Tuolumne, Mariposa (*Bolander*, no. 4958, type!, Yosemite Park, Slough's Valley), Fresno, Monterey, Tulare, and Kern. It may even occur farther south.

There is a specimen from San Bernardino County, near head of San Antonio Canyon, in a narrow rocky canyon, alt. 2250 m., July 5, 1918, *I. M. Johnston* (no. 2087, flor. abnorm. m. et f. mixtis; A.; "shrub, low, under 1 m."). The leaves are almost wholly glabrous when maturing, at least on the lower surface, which is more or less distinctly glaucescent. The flowers, however, are abnormal, the female ones hard to distinguish from those of *S. exigua*, but glabrous, or almost so. The form may belong to *S. exigua virens*, if there is really such a variety, or it may be related to var. *Bolanderiana*. The normal form is represented by *Johnston's* nos. 1401 and 1665, from the upper San Antonio Canyon. I am much obliged to Mr. JOHNSTON for the following information:

Numbers 1401, 1665, 2087 from near head of San Antonio Canyon. To me this is the most interesting plant I sent you. I have thoroughly explored the San Antonio Mountains, but I have only found the single colony from which all my specimens were obtained. It grows as a dense, low, compact shrub (hardly over a meter in height) on the rocky floor of a very deep gulch. A short distance away is found a large colony of *S. flavescens* and scattering shrubs of *S. Watsoni*. The nearest LONGIFOLIAE that I know of is 7 miles away and is the colony from which my 1685, which you doubtfully referred to *S. Parishiana*, was obtained. I have never yet seen in S. California a LONGIFOLIAE so high in the mountains and associating with such typically boreal species as this one does. You have probably noted that the aments contain both staminate and pistillate flowers, which may be due to its strange habitat. I noted that a large percentage of the aments were entirely sterile at the time I collected the specimens.

8. S. LONGIFOLIA Muhl. in Neue Schr. Ges. Natf. Fr. Berlin 4:238. *pl. 6. fig. 6.* 1803, non Lamarck;[11] in Ann. Bot. König

[11] According to the international rules, MUHLENBERG's name can stand because LAMARCK's (Fl. Fr. 2:232. 1778) is nothing but a synonym of *S. viminalis* L.; in following the Philadelphia Code the name *S. interior* Rowl. has to be used, and I would not keep MUHLENBERG's name if LAMARCK's were not an unconditional synonym, and could be applied to a form differing from typical *S. viminalis*.

2:66. *pl. 5. fig. 6.* 1806; Carey in Gray, Man. Bot. N.U.S. 429. 1848; Andersson in K. Sv. Vet.-Akad. Handl. 6:54. *pl. 4. fig. 35.* 1867, pro parte et excl. var.; in DC., Prodr. 16²:214. 1868, pro parte et excl. var.; Bebb in Coult., Man. Bot. R. Mts. 335. 1885, pro parte; apud Watson and Coulter, Gray Man. ed. 6. 482. 1890; Robinson and Fernald, Gray's New Man. 323. *fig. 649.* 1908.—*S. fluviatilis* Sargent in Gard. and For. 8:463. 1895, pro parte, non Nutt.; Silva N. Am. 9:123. *pl. 474.* 1896, pro parte et excl. var.; Man. Trees N. Am. 175. 1905, pro parte; Schneider, Ill. Handb. Laubh. I. 32, *figs. 11 h–l, 12 m–m¹.* 1904; Ball in Proc. Iowa Ac. Sci. 7:145. 1900; in Coult. and Nels., N. Man. R. Mts. Bot. 131. 1909, pro parte; in Bot. Gaz. 60:397. 1915; Britt. and Brown, Ill. Fl. 1:497. *fig. 1181.* 1896; Sudworth, Nomencl. Arb. Fl. U.S. 122. 1897, pro parte; Rydberg in Britt., Man. Fl. N. St. Can. 316. 1901; Hough, Handb. Trees N. St. Can. 84. *figs. 97, 98.* 1907, pro parte maxima.—*S. interior* Rowlee in Bull. Torr. Bot. Club 27:253. *pl. 9, figs. 12, 13.* 1900; Small, Fl. S.E.U.S. 342. 1903, pro parte; Britt. and Shafer, N. Am. Trees 193. *fig. 154.* 1908; Britt. and Brown, Ill. Fl. ed. 2. 1:595. *fig. 1458.* 1913; Rydberg, Fl. R. Mts. 192. 1917.—This is the type species of the section and the only one known from the central and northeastern states and eastern Canada. The type came from Lancaster, Pennsylvania. It has its headquarters in the regions of the Mississippi, Arkansas, and Missouri, while toward the east the Ohio seems to form the southern border line of its range up to Pennsylvania. The mouth of the Mississippi in Louisiana is the southernmost point of the range of *S. longifolia;* its western boundary runs apparently just south of the Red River in Louisiana and Texas, thence through western Kansas, the northeastern corner of Colorado, touching Wyoming in its northeastern part, from whence it runs through western Dakota to Manitoba. In Texas, southern New Mexico, and northwestern Mexico it is represented by var. *angustissima* (see later), while in the northwest from western Dakota and northeastern Wyoming through eastern Montana, Saskatchewan, and eastern Alberta the var. *pedicellata* seems to be the prevailing form, reaching its northwestern limit in the Yukon Valley (vicinity of Dawson and the adjacent parts of eastern Alaska, Fairbanks) and the upper Mackenzie region in the

Northwest Territories. The northern border line of the range of *S. longifolia* and var. *pedicellata* is not yet exactly known. Approximately it seems to run in the west from Fairbanks in Alaska to Fort Simpson in the Northwest Territories and through the Athabasca Plains and central (or southern?) Manitoba and southern Ontario to the south of James Bay and to about Lake St. Johns in Quebec, from where the eastern line turns southeast to western New Brunswick (Woodstock, Pokiok) and then southward to New Hampshire along the Connecticut River to Delaware and the District of Columbia.

The species apparently reaches its best development in the rich river bottoms from Louisiana to Indiana, while in Oklahoma, Kansas, Nebraska, and Iowa the form of the sand bars seems to prevail, which has narrower, smaller leaves. In the region of the Great Lakes and in the northeast, but also in other portions of the range under similar ecological conditions, the following variety seems to occur frequently:

S. LONGIFOLIA var. **Wheeleri,** nov. comb.—*S. interior* var. *Wheeleri* Rowlee in Bull. Torr. Bot. Club **27**:253, *pl. 9, fig. 14.* 1900. —*S. Wheeleri* Rydberg in Britt., Man. ed. 2. 1061. 1905; Britt. and Br., Ill. Fl. ed. 2. **1**:595. 1913.—*S. longifolia* (vel *S. fluviatilis*) var. *argyrophylla* Auct. div. pro parte, non And.—I agree to a certain extent with SCHAFFNER (in Ohio Nat. **14**:255. 1914), who regards this variety as an ecological form, and I have already pointed out that similar forms seem to occur in *S. exigua* (see var. *luteo-sericea*), *S. melanopsis* var. *Bolanderiana*, etc. Those forms very often look quite distinct, especially in the herbarium. The broad leaved forms of var. *Wheeleri* can easily be taken for a well marked species if one does not have a very rich set of specimens showing all the intermediates between such forms as we know from Maine (Caribou) and New Brunswick and the narrow leaved forms from Lake Champlain, Lake Superior, etc. It may be that the easternmost forms are not quite identical with the typical var. *Wheeleri* from the region of the Great Lakes, but to decide this question we need a careful study of this form as it is observed in New Brunswick, Maine, Connecticut, western Quebec, and eastern Ontario. There is a male plant in cultivation in the Arnold

Arboretum which was brought by Professor *J. G. Jack* probably from the St. Lawrence region in Ontario. It is extremely like the female specimen of *Bissell* from Glastonbury, Connecticut, and both agree well with the specimens cited from Maine and New Brunswick. In *Bissell's* plant the stigmas are rather long and narrow, resembling somewhat those of the western *S. fluviatilis* but without a trace of a style. The leaves too of both plants are not very different in their shape, but var. *Wheeleri* has a coarser silky pubescence of longer hairs. ROWLEE stated that "the silvery vesture of this shrub is much like that of *S. argophylla* of the Pacific Coast." As I have explained under this species, ROWLEE did not interpret it correctly.

At present I refer to var. *Wheeleri* the following specimens, and I hope collectors will pay attention to this plant at the localities given.

Eastern North Dakota: Benson County, Pleasant Lake, 99 mer., everywhere along watercourses, July 2, 1911, *J. Lunell* (m., flor. satis abnorm.; A.).—Iowa: Story County, Ames, 1888, *A. S. Hitchcock* (m.; M.); Fremont County, Hamburg, July 4, 1914, *L. H. Pammel* and *H. B. Clarke* (no. 44, m.; A.; a hairy sand-bar form).—Illinois: St. Clair County, Cahokia, July 23, 1895, *N. M. Glatfelder* (m.; M.); Winnebago County, Fountaindale, 1877, *M. S. Bebb*, (fr.; M.; narrow leaved form, probably cultivated); Cook County, Dunning, fields, May 16, 1916, *F. C. Gates* (no. 1428, m.; C.).—Indiana: Noble County, near Rome City, June 11, 1916, *Deam* (no. 20118 A, ex parte, f., fr.; A.); Union County, Liberty, July 1886, *J. N. Rose* (st.; C).—Michigan: Wayne County, Belle Isle, July 8, 1903, *D. A. Farwell* (f.; A.; according to a letter of FARWELL this form was named by ROWLEE himself var. *Wheeleri*, but it represents a very glabrescent form difficult to separate from typical *longifolia*).—Wisconsin: Brown County, Green Bay, south shore, June 1878, *J. H. Schuette* (m., st.; C.).—Minnesota: Buffalo Lake, June 1891, *B. C. Taylor*, (m.; C.).—Ohio: Erie County, Cedar Point, August 2, 1895, *E. L. Moseley* (st.; G.); September 4, 1898, *Moseley* (st.; W.; folia ad 8:2 m. magna, elliptico-oblonga); July 3, 1908, *R. F. Griggs* (no. 2, m.; N.; folia ad 8:1.5 cm. magna, distanter ciliato-serrata); without exact locality and date, *W. S. Sullivant* (no. 49, st.; N.); Lake County, near Painesville, May 19, 1892, *O. Hacker* (no. 431, m.; C.); Franklin County, Columbus, 1840, *W. S. S.* (st.; G.); Ottawa County, Bay Point, sandy shore, August 20, 1914, *L. H. MacDaniels* and *A. J. Eames* (fr.); Ross County, Chillicothe, June 16, 1899, *A. D. Selby* (no. 120, st.; C.).—Pennsylvania: Erie County, Presque Isle, Lake Erie, July 23, 1868, *T. C. Porter* (st.; N., C.); York County, shores of the Susquehanna near

McCall's Ferry, September 13, 1864, *T. C. Porter* (m.; C.; "shrub 5–6 ft. high"; forma peculiaris foliis late oblongo-ellipticis ad 9:2.2 cm. magnis).—New York: Erie County, shores of Lake Erie near Buffalo, June 30, 1899, *J. F. Cowell* (st.; N.); Clinton County, shore of Lake Champlain, near Plattsburg, August 8, 1902, *A. Rehder* (st.; A.); Tompkins County, Fall Creek ravine, on rocks, May 29, June 6, 1885, *W. R. Dudley* (m., st.; C.; folia pl.m. oblanceolata).—Vermont: wet shore of Lake Champlain, July 8, 1914, *Ch. H. Knowlton* (m.; NE.); June 15, 1896, *A. J. Grout* (f.; NE.; stigmata satis elongata).—Connecticut: Hartford County, Glastonbury, banks of Connecticut River, May 18, 1902, *C. H. Bissell* (f.; G.; "small shrub"; forma distincta porro observanda); New London County, Lyme, near Selden's Cove, July 29, 1902, *C. B. Graves* (st.; G.; "2 ft. high"; ut praecedens).—Maine: Aroostook County, Caribou, gravelly river beach, July 18, 1902, *E. F. Williams*, *J. F. Collins*, and *M. L. Fernald* (st.; G.; forma satis distincta porro observanda); same locality and date, *E. F. Williams* (st.; A., G.).—New Brunswick: Woodstock, on the bars in the St. John River, August 30, 1899, *Macoun* (no. 22609, O.; st.; very much like the Connecticut forms); near Pokiok, July 8, 1889, *Brittain* (no. 24577, O.; st.; ut praecedens); above Fredericton, on island, August 23, 1890, *J. Brittain* (no. 6, fr.; C.; ut praecedens); Keswick, June 6, 1891, *J. Brittain* (no. 4, f.; C.).—Ontario: Lambton County, Fort Frank, 35 miles from Port Huron, Michigan, July 21, 1905, *C. K. Dodge* (st.; A.; forma densissime sericea); Welland County, Point Albino, August 28, 1896, *C. L. Pollard* (st.; W.); James Bay, Moose Factory, July 15, 1904, *W. Spreadborough* (no. 6262e, O.; st.; forma porro observanda paullo sericea).

Every species inhabiting such a wide area as *S. longifolia* and growing under so many different ecological conditions will naturally show a great degree of variability. Besides this there are quasi intermediate forms with *S. exigua* in all the regions where both species meet, and it is difficult to decide whether the northwestern forms of what I call var. *pedicellata* really belong to *S. longifolia* or to *S. exigua*, as BALL seems to believe according to his determinations in different herbaria. The synonymy of var. *pedicellata* may be given as follows:

8b. S. LONGIFOLIA var. PEDICELLATA Andersson in K. Sv. Vet.-Akad. Handl. **6**:55. 1867; in DC., Prodr. **16**²:214. 1868.—*S. rubra* Richardson in Franklin, Narr. Jour. Polar Sea App. 752. 1823, nom. nud., non Hudson.—*S. longifolia* (?) Torrey in Ann. Lyc. Nat. Hist. N.Y. **2**:248 (Coll. Pl. R. Mts. James).[12] 1828; Andersson in

[12] The specimen (preserved in N.) has been collected by *James* either in eastern Wyoming or eastern Colorado, and seems to belong to this variety.

Öfv. K. Vet.-Akad. Förh. **15**:116. 1858, ex parte; Macoun, Cat. Canad. Pl. 450. 1883, ex parte; Sargent, Rep. For. Trees N. Am. 10th Census U.S. **9**:168. 1884, ex parte.—*S. fluviatilis* Sargent in Gard. and For. **8**:463. 1895, ex parte, non Nutt.; Rowlee in Bull. Torr. Bot. Club **27**:254. 1900, ex parte; Henry, Fl. S. Br. Col. 97. 1915.—*S. interior* Rowlee, l.c., 253, ex parte; Britt. and Br., Ill. Fl. **1**:595. 1913, ex parte.—*S. linearifolia* Rydbg. in Britton, Man. 316. 1901; Fl. Color. 94. 1906, ex parte; Fl. R. Mts. 192. 1917, ex parte; Small, Fl. S.E.U.S. 342. 1903, ex parte.—*S. longifolia* var. *interior* Jones, Willow Fam. 25. 1908, ex parte.—I have seen a photograph and fragments of the type of var. *pedicellata*, collected by *E. Bourgeau*, "Saskatchewan bords des Lacs, abondant, 21 Juin 1858" and preserved in Herb. K., and also of the type of *S. rubra* Rich. from the "Mackenzie River." This specimen of *Richardson's* represents the same form as the material from "Cumberland House" in Saskatchewan, which is a syntype of *S. linearifolia* Rydbg. in Herb. N. This variety differs from typical *S. longifolia* chiefly in its narrower, linear leaves, and its glabrous ovaries, which are more or less sessile when young but usually distinctly pediceled when in fruit, the pedicels often being twice as long as the ventral gland. As previously stated, var. *pedicellata* is the prevailing form in the northwestern part of the range of *S. longifolia*, but there are also forms near the southern limit of its habitat which can hardly be distinguished from var. *pedicellata* (for instance *Munson's* specimens from the Red River near Colbert's Ferry, north of Denison, Texas, April 19, 1911, f., fr.; A.).

As previously stated, the most southern form of *S. longifolia* is represented by var. *angustissima* And. (1858[13]) with which I have dealt in Bot. Gaz. **65**:26. 1918. Besides the Mexican specimens here cited, I refer the following to this variety, which seems too closely connected with the typical *S. longifolia* to be kept as a distinct species.

Specimens examined.—Texas: without exact locality and date, *Berlandier* (nos. 911, 2341, 2368, 3019, cotypes; G., M.; 1938, f.; M.; nos. 2341 and

[13] Later, in Monogr. 1867 and in Prodr. 1868, Andersson used this name for different forms, partly belonging to *S. sessilifolia* var. *Hindsiana*, partly to *S. exigua* (probably var. *stenophylla*).

2368, of which the last has to be taken as the type of *S. Thurberi*, have been erroneously attributed by ROWLEE to *G. Thurber*, to whom only the following specimen belongs); Horse Head Cruping (?) River, October 1850, *G. Thurber* (no. 95; G.; "10–12 ft."); ?Pecos County, banks of the Pecos, 1889, *Nealhy* (no. 33, m.; W.); September 1881, *V. Havard* (m., f.; W.; ad var. *typicam* accedens); Brewster County, Rio Grande, south of Chisos Mountains, August 1883, *V. Havard* (m., f.; W.); Val Verde County, Del Rio, along streams, October 18, 1916, *E. J. Palmer* (no. 11069, f.; A.); Potter County, Amarillo, creek banks, July 13, 1917, *E. J. Palmer* (no. 12539, f., fr.; A.; ad var. typicam accedens); along Rio Grande, near San Vincente, August 26, 1915, *M. S. Young* (m., f.; M.); Guadalupe County, in the dry bed of the Cibolo 12 miles east of New Braunfels, August 1851, *F. Lindheimer* (no. 615 [=1191], f.; G., M.); Comanche County, Comanche Spring, *Lindheimer* (no. 1190, f.; M.); Matagorda County, banks of Peyton Creek near Bay City, May 6, 1916, *E. J. Palmer* (no. 9689, m.; A.); Cameron County, near Brownsville, November 1888, *Nealhy* (no. 30, f., fr.; W.); (New Mexico?), Rio Grande, July 1848, *C. Wright* (m.; G.; "small tree"); without locality, 1849, *C. Wright* (no. 668, m.; G., W.).

There have also been described the following forms which I have not yet been able to elucidate: *S. longifolia* var. *sericans* Nees v. Esenbeck in Wied-Neuwied, Reise In. N. Am. 2:448. 1841; Engl. ed. by Lloyd, Trav. Int. N.A. 518. 1843, collected on the Missouri, probably in eastern Montana about July 8 (see *l.c.* 1:472 [Engl. ed. p. 211]). I would refer it to *S. exigua*, but the lower flowers of the male aments are described as "triandri"; otherwise the description agrees with *S. exigua*.—*S. longifolia* f. *integerrima* Kuntze, Rev. Gen. Pl. 2:643. 1891, and f. *paucidenticulata* Kuntze, l.c. The first is characterized by the phrase "folia denticulata" and as type is given "U. St., Madisonthal"; while the second has "folia paucidenticulata" and came from "Cheyenne, Nebr." The author adds "Ausserdem kann man eine f. *multidenticulata* unterscheiden." I suppose those forms are simply typical *S. longifolia*.

With the hybrids which doubtless occur only too frequently where different species grow together it is impossible to deal, as long as it has not yet been possible to limit the species in a more satisfactory manner. The main purpose of this paper is to point out the correct application of certain names, and to direct attention to such forms as need a close study in the field.

ARNOLD ARBORETUM
JAMAICA PLAIN, MASS.

RESPIRATION AFTER DEATH[1]

A. R. C. Haas

(WITH THREE FIGURES)

It is commonly stated that when respiration ceases the protoplasm is no longer alive, but it is uncertain in most cases whether respiration ends as soon as death occurs or whether it continues for some time afterward.

It was stated by Johannsen (8), by Detmer (6), and by Pfeffer (17) that in general there is no production of CO_2 after death, although Reinke (18) and Brenstein (3) held the opposite view. Buchner (4) showed that yeast which had been treated with acetone and ether and which was incapable of cell division, and in all probability dead, could produce CO_2 by fermentation. Kostytscheff (10) found that an aërobic plant, *Aspergillus niger*, treated in this manner was still capable of respiration. Since some of the cells appeared to be alive after the treatment, he used heat to kill them. After this the oxidation was extremely small. This is to be expected as the oxidases are, for the most part, injured or destroyed by heat. Similar experiments have been made on bacteria. Warburg (21) obtained a completely sterile acetone preparation of *Staphylococcus* which respired about one thirty-sixth as much as the living material. Warburg and Meyerhof (21) found that treatment with acetone and ether had little effect on the consumption of oxygen by unfertilized sea urchin eggs (although they were completely killed), but the same treatment diminished the consumption of oxygen by fertilized eggs by 90 per cent.

Numerous experiments have been made with cells killed by mechanical means (finely ground) or by freezing and thawing. Palladin (16) found that finely ground wheat produced less CO_2 than the normal amount, while various plants exposed to $-20°$ C. for some time and then thawed out showed a loss of power to absorb oxygen, but continued to produce CO_2.

[1] A preliminary communication appeared in Proc. Nat. Acad. Sci.

BATELLI and STERN (2) have found oxidation in finely ground tissue and watery extracts. Their results have been criticized by WARBURG.

WARBURG (21) found that the finely ground red blood corpuscles of birds consumed less oxygen than intact cells. Unfertilized sea urchin eggs, cytolyzed in distilled water, consumed as much oxygen as the intact eggs but produced no CO_2. In fertilized eggs cytolysis reduced the oxygen consumption by 90 per cent or more. A fuller account of the literature seems unnecessary, as it has been summarized by WARBURG.

It will be noticed that in the cases previously reported respiration after death is greatly reduced or entirely lacking. The only instance in which post mortem respiration is greater than in normal tissue is that reported by LOEB and WASTENEYS (11), in which unfertilized sea urchin eggs, cytolyzed by saponin, showed from 3 to 7 times the normal rate of respiration. It is of considerable interest therefore to find that the respiration of *Laminaria* after death may be much greater than when in its normal condition.

The determination of the output of CO_2 was made in the following manner. The increase in the hydrogen ion concentration of sea water containing pieces of *Laminaria* (in the dark) served as a measure of the respiration of the tissue. The decrease in PH value was determined by the addition of a suitable indicator (phenolsulphone phthalein) by comparing the colors with those of a series of buffer mixtures containing an equal amount of the same indicator.

Each piece of *Laminaria* was kept for about half an hour in sea water before beginning the experiment. This treatment tended to obviate any effects of the slight wounding (19, 20). The material was then rolled into a scroll and inserted into a Pyrex glass tube (7) fused shut at one end and attached to a paraffined rubber tube at the open end. Sea water, of the temperature of the bath (16 ± 1°C.), was placed in the tube and the latter inserted in a black enameled collapsible tin tube in the bath. The sea water surrounding the tissue (in the tube) was renewed several times before beginning the experiment. A definite amount of sea water

(6 cc.) was placed in each of the tubes. The tubes were clamped shut in such a way as to include a very small air bubble (always of the same size) which served as a stirrer. This was sufficiently accurate and was more convenient than paraffined glass beads. After a tube had been in the dark at 16± 1°C. for a definite period, it was removed from the bath, and the contents shaken by inverting the tube several times. The sea water was then poured rapidly into an empty tube of equal diameter, to which the same quantity (3 drops of 0.01 per cent aqueous phenolsulphone phthalein to 6 cc. of solution) of indicator was added as had been added to the buffer mixtures. The color was then compared with the colors of a series of buffer mixtures by the use of a constant source of light (the "Daylight" lamp) and the PH value determined. The same amount of sea water was again added to the tissue in the tube and the tube exposed (at 16± 1°C. in the dark) for the same length of time as before, after which it was removed from the bath and the PH value again determined. This was repeated until the respiration in sea water was approximately constant. Then sea water containing the killing agent was substituted for the sea water, and the PH values determined as before after a series of successive periods (each of the same length as the original).

In some cases (acetone 17.4 and alcohol 24.2 per cent) the killing agent extracted from the plant a small amount of pigment which interfered with the color of the indicator.[2] This difficulty disappeared after the first two periods, however, as was shown by running pure hydrogen through the solution, after which it returned to the color found in normal sea water containing indicator. This method also showed conclusively that the only acid excreted by the plant was carbonic acid.

The methods of killing the tissue were various. Sea water containing anesthetics (made up to the conductivity of sea water by the addition of concentrated sea water) was employed in many of the experiments. In this case the respiration was determined for several periods of equal length in sea water (the solution being renewed after each period). The sea water was then replaced by

[2] This did not occur with low concentrations of these substances.

sea water containing anesthetic and the respiration determined after successive equal periods until death ensued, and for some time thereafter.

TABLE I

CONTROL FOR I A: 7 PERIODS (27.5 MIN. EACH) IN SEA WATER; SOLUTION RENEWED AT BEGINNING OF EACH PERIOD

Period	Change in PH	Total change in PH = A	Change in PH calculated from first 2 periods = B	Relative amount of respiration = A/B	Time in min.	Relative rate of respiration
1.....	8.1 − 7.6 = 0.5	0.5	0.5	1.00	27.5	0.5 ÷ 0.5 = 1.00
2.....	8.1 − 7.6 = 0.5	1.0	1.0	1.00	55.0	0.5 ÷ 0.5 = 1.00
3.....	8.1 − 7.6 = 0.5	1.5	1.5	1.00	82.5	0.5 ÷ 0.5 = 1.00
4.....	8.1 − 7.6 = 0.5	2.0	2.0	1.00	110.0	0.5 ÷ 0.5 = 1.00
5.....	8.1 − 7.65 = 0.45	2.45	2.5	0.98	137.5	0.45 ÷ 0.5 = 0.90
6.....	8.1 − 7.65 = 0.45	2.90	3.0	0.96	165.0	0.45 ÷ 0.5 = 0.90
7.....	8.1 − 7.65 = 0.45	3.35	3.5	0.96	192.5	0.45 ÷ 0.5 = 0.90

TABLE I A

CHANGE IN PH VALUE OF SEA WATER PRODUCED BY RESPIRATION OF *Laminaria* DURING 2 PERIODS (22 MIN. EACH) IN SEA WATER AND DURING 7 SUBSEQUENT PERIODS IN SEA WATER APPROXIMATELY SATURATED WITH ETHYL BROMIDE

Solution	Period	Change in PH	Change in PH during saturation with ethyl bromide = A	Change in PH calculated from first 2 periods = B	Relative amount of respiration = A/B	Time in min.	Time during saturation with ethyl bromide in min.	Relative rate of respiration
Sea water.....	1	8.1 − 7.8 = 0.30	.....	.....	.....	22	0	.....
" "	2	8.1 − 7.8 = 0.30	.....	.....	.....	44	0	.....
Sea water containing ethyl bromide.....	3	7.32 − 6.16* = 1.16+	1.16	0.30	3.8	66	22	1.16 ÷ 0.30 = 3.8
Sea water containing ethyl bromide.....	4	7.32 − 6.16* = 1.16+	2.32	0.60	3.8	88	44	1.16 ÷ 0.30 = 3.8
Sea water containing ethyl bromide.....	5	7.32 − 6.69 = 0.63	2.95	0.90	3.2	110	66	0.63 ÷ 0.30 = 2.1
Sea water containing ethyl bromide.....	6	7.32 − 7.07 = 0.25	3.20	1.20	2.6	132	88	0.25 ÷ 0.30 = 0.8
Sea water containing ethyl bromide.....	7	7.32 − 7.21 = 0.11	3.31	1.50	2.2	154	110	0.11 ÷ 0.30 = 0.4
Sea water containing ethyl bromide.....	8	7.32 − 7.32 = 0.0	3.31	1.80	1.8	176	132	0.0 ÷ 0.30 = 0.0

* Approximate (at this point indicator is not very sensitive to slight changes in acidity).

As it was important to know the time of death as accurately as possible, determinations of the electrical conductivity of the tissue were made by the method of OSTERHOUT (12, 13). If the

electrical resistance of the normal tissue be called 100 per cent, it is found that after killing the resistance falls to about 10 per cent. When the resistance has fallen to 15 per cent the tissue is for all practical purposes dead, as there is no recovery when it is returned to normal conditions.[3]

The results of the experiments showing the relative amount and relative rate of respiration of tissue of *Laminaria* when subjected to various treatments are presented in tabular form. In every case 6–12 or more closely agreeing results were obtained and the data

TABLE I B

NET ELECTRICAL RESISTANCE OF *Laminaria* IN SEA WATER AND IN SEA WATER APPROXIMATELY SATURATED WITH ETHYL BROMIDE, EXPRESSED AS PERCENTAGE OF NET RESISTANCE AT START OF EXPERIMENT AT 20° C.

SEA WATER		SEA WATER APPROXIMATELY SATURATED WITH ETHYL BROMIDE	
Time in min.	Percentage net resistance	Time in min.	Percentage net resistance
0.....	100	0.....	100
100.....	90.9	1.....	105
200.....	87.8	5.....	79
		10.....	56
		15.....	39
		20.....	30
		35.....	16
		60.....	11
		80.....	10
		1000.....	5

of a typical case presented. Table I A shows the relative amount and relative rate of respiration as influenced by sea water approximately saturated with ethyl bromide. This is the only experiment in which the PH value of the control differed from the PH value of the sea water containing anesthetic (at the start of the experiment). It will be noted that after about 130 minutes no respiration was detected. An examination of table I B, which gives the net electrical resistance of *Laminaria*, shows that the material can be considered dead before the end of 60 minutes. From table I A it is seen that at the end of 60 minutes the relative rate and relative

[3] The determinations were made in part by Professor OSTERHOUT and in part by me.

amount of respiration are approximately double that of the normal, although the tissue is shown by the method of electrical resistance to be dead. This is brought out very strikingly in fig. 1 where we plot as ordinates the relative amount of respiration (curve C, table I A), relative rate of respiration (curve B, table I A), net resistance as percentage of that at the start (curve A, table I B) respectively (unbroken lines). When the relative rate of respiration has practically reached zero (curve B) the relative amount of

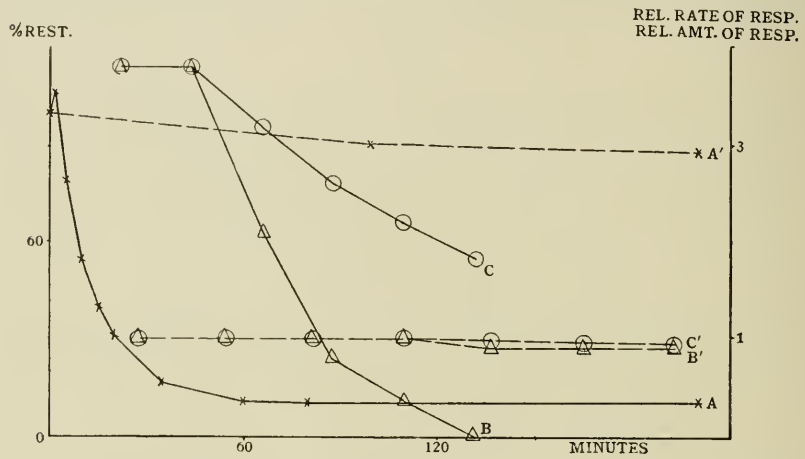

FIG. 1.—Curves showing effect produced by sea water, approximately saturated with ethyl bromide, upon relative amount and relative rate of respiration, and upon net electrical resistance of *Laminaria:* curve A, ordinates represent net resistance as percentage of that at start; curve B, ordinates represent relative rate of respiration; curve C, ordinates represent relative amount of respiration (unbroken lines); controls in sea water (broken lines); each control curve bears same symbol and letter (with a prime) as experimental curve; abscissae represent time in minutes.

respiration is above unity. At the end of 60 minutes, when the tissue can be considered dead, the relative rate is seen to be about double that of the normal rate.

Table II A shows the effect produced by sea water containing 17.4 per cent (by volume) acetone, made up to the electrical conductivity of sea water by the addition of concentrated sea water, upon the relative amount and relative rate of respiration of *Laminaria.* At the end of 2.5 hours the rate of respiration is still above the normal rate, while the relative amount of respira-

tion is nearly 2. Table II B shows the net electrical resistance. It is seen that the material is dead before the end of 100 minutes.

TABLE II

CONTROL FOR II A: 9 PERIODS (21 MIN. EACH) IN SEA WATER; SOLUTION RENEWED AT BEGINNING OF EACH PERIOD

Period	Change in PH	Total change in PH = A	Change in PH calculated from first 2 periods = B	Relative amount of respiration = A/B	Time in min.	Relative rate of respiration
1.....	8.37−7.67=0.70	0.70	0.70	1.0	21	0.70÷0.70=1.0
2.....	8.37−7.67=0.70	1.40	1.40	1.0	42	0.70÷0.70=1.0
3.....	8.37−7.70=0.67	2.07	2.10	0.99	63	0.67÷0.70=0.95
4.....	8.37−7.73=0.64	2.71	2.80	0.97	84	0.64÷0.70=0.91
5.....	8.37−7.73=0.64	3.35	3.50	0.96	105	0.64÷0.70=0.91
6.....	8.37−7.73=0.64	3.99	4.20	0.95	126	0.64÷0.70=0.91
7.....	8.37−7.72=0.65	4.64	4.90	0.95	147	0.65÷0.70=0.93
8.....	8.37−7.73=0.64	5.28	5.60	0.94	168	0.64÷0.70=0.91
9.....	8.37−7.72=0.65	5.93	6.30	0.94	189	0.65÷0.70=0.93

TABLE II A

CHANGE IN PH VALUE OF SEA WATER PRODUCED BY RESPIRATION OF *Laminaria* DURING 2 PERIODS (24 MIN. EACH) IN SEA WATER, AND DURING 7 SUBSEQUENT PERIODS IN SEA WATER CONTAINING 17.4 PER CENT (BY VOLUME) OF ACETONE

Solution	Period	Change in PH	Change in PH during exposure to acetone = A	Change in PH calculated from first 2 periods = B	Relative amount of respiration = A/B	Time in min.	Time of exposure to acetone in min.	Relative rate of respiration
Sea water.....	1	8.37−7.76=0.61	.....	.....	.....	24	0	.....
" "	2	8.37−7.78=0.59	.....	.....	.....	48	0	.....
Sea water containing 17.4 per cent acetone	3	8.37−6.80=1.57	1.57	0.60	2.61	72	24	1.57÷0.60=2.6
Sea water containing 17.4 per cent acetone	4	8.37−6.75=1.62	3.19	1.20	2.66	96	48	1.62÷0.60=2.7
Sea water containing 17.4 per cent acetone	5	8.37−7.06=1.31	4.50	1.80	2.50	120	72	1.31÷0.60=2.2
Sea water containing 17.4 per cent acetone	6	8.37−7.50=0.87	5.37	2.40	2.24	144	96	0.87÷0.60=1.5
Sea water containing 17.4 per cent acetone	7	8.37−7.54=0.83	6.20	3.00	2.07	168	120	0.83÷0.60=1.4
Sea water containing 17.4 per cent acetone	8	8.37−7.62=0.75	6.95	3.60	1.93	192	144	0.75÷0.60=1.3
Sea water containing 17.4 per cent acetone	9	8.37−7.74=0.63	7.58	4.20	1.80	216	168	0.63÷0.60=1.1

The fact that respiration proceeds here at a rate much above the normal (although death has taken place) is very clearly brought

out by comparing the curves for table II A and II B as given in fig. 2. The ordinates represent relative amount of respiration (curve A, table II A), relative rate of respiration (curve C, table II A), net resistance as percentage of that at the start (curve B, table II B) respectively (unbroken lines). The relative rate and relative amount of respiration at the end of over 2.5 hours are still much above the normal even though the measurements of electrical resistance have shown the tissue to be dead before 100 minutes.

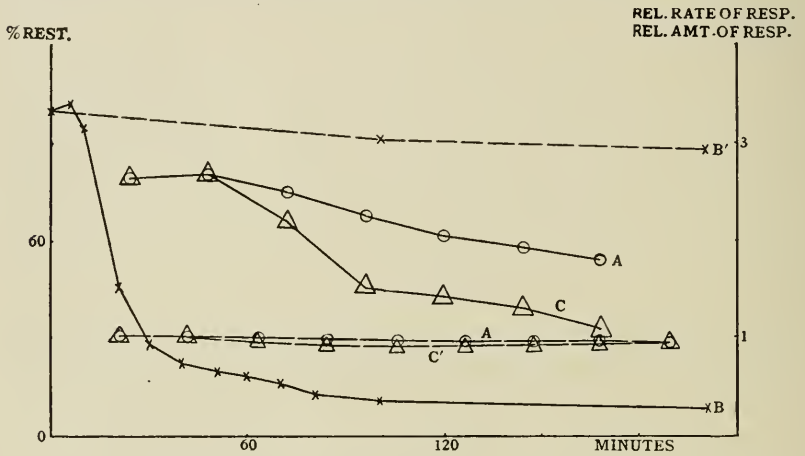

FIG. 2.—Curves showing effect produced by sea water containing 17.4 per cent (by volume) of acetone upon relative amount and relative rate of respiration, and effect produced by sea water containing 16.2 per cent of acetone upon net electrical resistance of *Laminaria:* curve A, ordinates represent relative amount of respiration; curve B, ordinates represent net resistance as percentage of that at start; curve C, ordinates represent relative rate of respiration (unbroken lines); controls in sea water (broken lines); each control curve bears same symbol and letter (with a prime) as experimental curve; abscissae represent time in minutes.

Table III A shows the effect produced by sea water containing 24.2 per cent (by volume) of ethyl alcohol (made up to conductivity of sea water by the addition of concentrated sea water). In fig. 3 the ordinates represent: relative amount of respiration (curve A, table III A), relative rate of respiration (curve C, table III A), net resistance as percentage of that at the start (curve B, table III B) respectively (unbroken lines). If we consider the material dead at the end of 90 minutes, we find that the

rate of respiration is much above the normal rate, while the relative amount of respiration is above 2.

TABLE II B

NET ELECTRICAL RESISTANCE OF *Laminaria* IN SEA WATER AND IN SEA WATER CONTAINING 16.2 PER CENT (BY VOLUME) OF ACETONE, EXPRESSED AS PERCENTAGE OF NET RESISTANCE AT START OF EXPERIMENT AT 15.4° C.

SEA WATER		SEA WATER CONTAINING 16.2 PER CENT ACETONE	
Time in min.	Percentage net resistance	Time in min.	Percentage net resistance
0.....	100	0.....	100
100.....	90.9	5.....	105.5
200.....	87.8	10.....	94.3
		20.....	46.6
		30.....	28.4
		40.....	22.7
		50.....	20.2
		60.....	18.2
		70.....	16.2
		80.....	13.2
		100.....	11.1
		200.....	8.7
		300.....	6.7

TABLE III

CONTROL FOR III A: 8 PERIODS (30.25 MIN. EACH) IN SEA WATER; SOLUTION RENEWED AT BEGINNING OF EACH PERIOD

Period	Change in PH	Total change in PH = A	Change in PH calculated from first 2 periods = B	Relative amount of respiration = A/B	Time in min.	Relative rate of respiration
1.....	7.90−7.53=0.37	0.37	0.37	1.0	30.25	0.37÷0.37=1.0
2.....	7.90−7.53=0.37	0.74	0.74	1.0	60.50	0.37÷0.37=1.0
3.....	7.90−7.53=0.37	1.11	1.11	1.0	90.75	0.37÷0.37=1.0
4.....	7.90−7.54=0.36	1.47	1.48	0.99	121.00	0.36÷0.37=0.97
5.....	7.90−7.54=0.36	1.83	1.85	0.99	151.25	0.36÷0.37=0.97
6.....	7.90−7.55=0.35	2.18	2.22	0.98	181.50	0.35÷0.37=0.95
7.....	7.90−7.55=0.35	2.53	2.59	0.97	211.75	0.35÷0.37=0.95
8.....	7.90−7.58=0.32	2.85	2.96	0.96	242.00	0.32÷0.37=0.87

Table IV shows the effect produced upon the relative amount and relative rate of respiration of *Laminaria* by sea water containing 3.2 per cent (by volume) of formaldehyde. The solution was

made up to the conductivity of sea water by the addition of concentrated sea water. The free acid of the formaldehyde was first neutralized by the addition of a little sodium carbonate. This is allowable for the purposes of the present investigation, for its effect would be to make the amount of CO_2 produced appear somewhat less than was actually the case. At the end of 4 hours the relative rate of respiration was still above the normal, while the

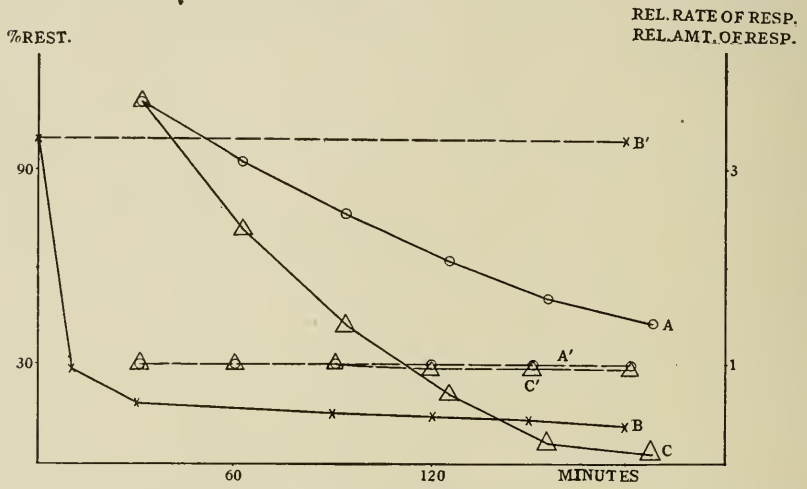

FIG. 3.—Curves showing effect produced by sea water containing 24.2 per cent (by volume) of ethyl alcohol upon relative amount and relative rate of respiration and upon net electrical resistance of *Laminaria:* curve A, ordinates represent relative amount of respiration; curve B, ordinates represent net resistance as percentage of that at start; curve C, ordinates represent relative rate of respiration (unbroken lines); controls in sea water (broken lines); each control curve bears same symbol and letter (with a prime) as experimental curve; abscissae represent time in minutes.

relative amount of respiration was much above the normal. At this concentration of formaldehyde *Laminaria* is practically dead in 180 minutes. In table IV, however, after 280 minutes the relative rate of respiration of *Laminaria* is still above normal, while at 180 minutes the relative rate is far above normal.

For purposes of comparison other methods of killing were tried. By making preliminary conductivity experiments with *Laminaria*, it was found that when it is dried upon cheesecloth in the sunlight in a current of dry air, we can consider the tissue practically dead

in 135 minutes. After such treatment the material becomes green. It was placed in sea water for 14 minutes; it lost its crispness and

TABLE III A

CHANGE IN PH VALUE OF SEA WATER PRODUCED BY RESPIRATION OF *Laminaria* DURING 2 PERIODS (31.25 MIN. EACH) IN SEA WATER AND DURING 6 SUBSEQUENT PERIODS IN SEA WATER CONTAINING 24.2 PER CENT (BY VOLUME) OF ETHYL ALCOHOL

Solution	Period	Change in PH	Change in PH during the 6 later periods = A	Change in PH calculated from first 2 periods = B	Relative amount of respiration = A/B	Time in min.	Time (in min.) of exposure during the 6 later periods	Relative rate of respiration
Sea water	1	7.90−7.43 =0.47	.....	.....	.....	31.25	0	.....
" "	2	7.90−7.43 =0.47	.....	.....	.....	62.50	0	.....
Sea water containing 24.2 per cent ethyl alcohol..	3	7.90−6.16*=1.74	1.74	0.47	3.57	93.75	31.25	1.74÷0.47=3.7
Sea water containing 24.2 per cent ethyl alcohol..	4	7.90−6.75 =1.15	2.89	0.94	3.07	125.00	62.50	1.15÷0.47=2.4
Sea water containing 24.2 per cent ethyl alcohol..	5	7.90−7.25 =0.65	3.54	1.41	2.51	156.25	93.75	0.65÷0.47=1.4
Sea water containing 24.2 per cent ethyl alcohol..	6	7.90−7.55 =0.35	3.89	1.88	2.07	187.50	125.00	0.35÷0.47=0.7
Sea water containing 24.2 per cent ethyl alcohol..	7	7.90−7.80 =0.10	3.99	2.35	1.69	218.75	156.25	0.10÷0.47=0.2
Sea water containing 24.2 per cent ethyl alcohol..	8	7.90−7.84 =0.06	4.05	2:82	1.43	250.00	187.50	0.06÷0.47=0.1

* Approximate (at this point the indicator is not very sensitive to changes in PH).

TABLE III B

NET ELECTRICAL RESISTANCE OF *Laminaria* IN SEA WATER AND IN SEA WATER CONTAINING 24 PER CENT (BY VOLUME) OF ETHYL ALCOHOL, EXPRESSED AS PERCENTAGE OF NET RESISTANCE AT START OF EXPERIMENT AT 13.3° C.

SEA WATER		SEA WATER CONTAINING 24 PER CENT ETHYL ALCOHOL	
Time in min.	Percentage net resistance	Time in min.	Percentage net resistance
0.....	100	0.....	100
240.....	98	10.....	28.8
		30.....	18.6
		90.....	15.2
		120.....	14.9
		150.....	13.5
		180.....	11.8
		210.....	11.3

became flaccid. The relative amount and relative rate of respiration of pieces of *Laminaria* were determined before and after the

drying treatment (which killed the tissue). The results are given in table V A, the control data being given in table V. The relative amount of respiration after the treatment was very high and after 2 hours was still at 3. The relative rate of respiration after the treatment was very high at the start but gradually declined to normal in 2 hours.

TABLE IV

CONTROL FOR IV A: 15 PERIODS (21 MIN. EACH) IN SEA WATER; SOLUTION RENEWED AT BEGINNING OF EACH PERIOD

Period	Change in PH	Total change in PH = A	Change in PH calculated from first 2 periods = B	Relative amount of respiration = A/B	Time in min.	Relative rate of respiration
1.....	8.37−7.80=0.57	0.57	0.52	1.1	21	0.57÷0.52=1.10
2.....	8.37−7.90=0.47	1.04	1.04	1.0	42	0.47÷0.52=0.90
3.....	8.37−7.90=0.47	1.51	1.56	0.96	63	0.47÷0.52=0.90
4.....	8.37−7.90=0.47	1.98	2.08	0.95	84	0.47÷0.52=0.90
5.....	8.37−7.92=0.45	2.43	2.60	0.93	105	0.45÷0.52=0.87
6.....	8.37−7.92=0.45	2.88	3.12	0.92	126	0.45÷0.52=0.87
7.....	8.37−7.92=0.45	3.33	3.64	0.92	147	0.45÷0.52=0.87
8.....	8.37−7.92=0.45	3.78	4.16	0.90	168	0.45÷0.52=0.87
9.....	8.37−7.90=0.47	4.25	4.68	0.90	189	0.47÷0.52=0.90
10.....	8.37−7.90=0.47	4.72	5.20	0.91	210	0.47÷0.52=0.90
11.....	8.27−7.90=0.47	5.19	5.72	0.91	231	0.47÷0.52=0.90
12.....	8.37−7.90=0.47	5.66	6.24	0.91	252	0.47÷0.52=0.90
13.....	8.37−7.90=0.47	6.13	6.76	0.91	273	0.47÷0.52=0.90
14.....	8.37−7.90=0.47	6.60	7.28	0.91	294	0.47÷0.52=0.90
15.....	8.37−7.90=0.47	7.07	7.80	0.91	315	0.47÷0.52=0.90

Another method used in killing the tissue was by placing it in running tap water. A preliminary determination of the electrical resistance showed that 22 hours were more than sufficient to kill the tissue. The experiment was begun at 11:18 A.M. and at 7:50 P.M. the tissue was still somewhat alive, but at 9:25 A.M. next day the tissue had in all probability been dead for some time. The respiration was then determined before and after exposure to running tap water for 19 hours. The results are given in table VI, the data for the control being given in table V A. In table VI it is obvious that no respiration of the tissue was observable after it had been in tap water for 19 hours. There is of course the possibility that the rise and decline of the respiration after death was so rapid as to escape observation if the tissue had been dead much before the end of 19 hours.

TABLE IV A

CHANGE IN PH VALUE OF SEA WATER PRODUCED BY RESPIRATION OF *Laminaria* DURING 2 PERIODS (23.5 MIN. EACH) IN SEA WATER AND DURING 13 SUBSEQUENT PERIODS IN SEA WATER CONTAINING 3.2 PER CENT FORMALDEHYDE

Solution	Period	Change in PH	Change in PH during exposure to formaldehyde = A	Change in PH calculated from first 2 periods = B	Relative amount of respiration = A/B	Time in min.	Time of exposure to formaldehyde in min.	Relative rate of respiration
Sea water	1	8.37−7.82=0.55	.....	.....	.....	23.5	0	.....
" "	2	8.37−7.82=0.55	.....	.....	.....	47.0	0	.....
Sea water containing 3.2 per cent formaldehyde	3	8.37−6.90=1.47	1.47	0.55	2.67	70.5	23.5	1.47÷0.55=2.7
Sea water containing 3.2 per cent formaldehyde	4	8.37−6.90=1.47	2.94	1.10	2.67	94.0	47.0	1.47÷0.55=2.7
Sea water containing 3.2 per cent formaldehyde	5	8.37−7.05=1.32	4.26	1.65	2.58	117.5	70.5	1.32÷0.55=2.4
Sea water containing 3.2 per cent formaldehyde	6	8.37−7.15=1.22	5.48	2.20	2.49	141.0	94.0	1.22÷0.55=2.2
Sea water containing 3.2 per cent formaldehyde	7	8.37−7.33=1.04	6.52	2.75	2.37	164.5	117.5	1.04÷0.55=1.9
Sea water containing 3.2 per cent formaldehyde	8	8.37−7.50=0.87	7.39	3.30	2.24	188.0	141.0	0.87÷0.55=1.6
Sea water containing 3.2 per cent formaldehyde	9	8.37−7.55=0.82	8.21	3.85	2.13	211.5	164.5	0.82÷0.55=1.5
Sea water containing 3.2 per cent formaldehyde	10	8.37−7.58=0.79	9.00	4.40	2.04	235.0	188.0	0.79÷0.55=1.4
Sea water containing 3.2 per cent formaldehyde	11	8.37−7.60=0.77	9.77	4.95	1.97	258.5	211.5	0.77÷0.55=1.4
Sea water containing 3.2 per cent formaldehyde	12	8.37−7.65=0.72	10.49	5.50	1.90	282.0	235.0	0.72÷0.55=1.3
Sea water containing 3.2 per cent formaldehyde	13	8.37−7.70=0.67	11.16	6.05	1.84	305.5	258.5	0.67÷0.55=1.2
Sea water containing 3.2 per cent formaldehyde	14	8.37−7.80=0.57	11.73	6.60	1.77	329.0	282.0	0.57÷0.55=1.0
Sea water containing 3.2 per cent formaldehyde	15	8.37−7.85=0.52	12.25	7.15	1.71	352.5	305.5	0.52÷0.55=0.95

TABLE V

CONTROL FOR V A: 3 PERIODS (25.75 MIN. EACH) IN SEA WATER; BETWEEN SECOND AND THIRD PERIODS AN INTERVAL OF 19 HOURS DURING WHICH TISSUE WAS BATHED IN RUNNING SEA WATER

Period	Change in PH	Total change in PH = A	Change in PH calculated from first 2 periods = B	Relative amount of respiration = A/B	Time in min.	Relative rate of respiration
1.....	8.0−7.58=0.42	0.42	0.42	1.00	25.75	0.42÷0.42=1.00
2.....	8.0−7.58=0.42	0.84	0.84	1.00	51.50	0.42÷0.42=1.00
3.....	8.0−7.60=0.40	1.24	1.26	0.98	77.25	0.40÷0.42=0.95

By determining the electrical resistance it was found that *Laminaria* is killed by exposure to 35° C. for 70 minutes. The respiration before and after such exposure was then determined. During the treatment at 35° C. the material was removed from the tubes and placed in a large volume of the sea water kept at 35° C. The results are given in table VII A. After the exposure to 35° C., the relative amount and rate of respiration had fallen considerably below the normal. This might be expected on the ground that oxidizing enzymes are injured or destroyed by heat.

TABLE V A

CHANGE IN PH VALUE OF SEA WATER PRODUCED BY RESPIRATION OF *Laminaria* DURING 6 PERIODS (30.5 MIN. EACH) IN SEA WATER; AT END OF SECOND PERIOD MATERIAL DRIED IN CURRENT OF AIR IN SUN FOR 139 MINUTES; MATERIAL THEN PLACED IN SEA WATER AT 22° C. FOR 15 MINUTES BEFORE BEGINNING THIRD PERIOD.

Period	Change in PH	Change in PH after drying = A	Change in PH before drying, calculated from first 2 periods = B	Relative amount of respiration = A/B	Time in min.	Time (in. min.) of exposure after drying	Relative rate of respiration
1.....	8.15−8.0 =0.15	.....	.....	.....	30.5	0	.....
2.....	8.15−8.0 =0.15	.....	.....	.....	61.0	0	.....
3.....	8.15−7.20=0.95	0.95	0.15	6.3	91.5	30.5	0.95÷0.15=6.3
4.....	8.15−7.70=0.45	1.40	0.30	4.6	122.0	61.0	0.45÷0.15=3.0
5.....	8.15−7.85=0.30	1.70	0.45	3.8	152.5	91.5	0.30÷0.15=2.0
6.....	8.15−8.00=0.15	1.85	0.60	3.1	183.0	122.0	0.15÷0.15=1.0

It is well known that severe injury causes a considerable rise in the respiration, and it seemed desirable to make such experiments with *Laminaria*. After the normal respiration of a piece of tissue had been determined, the material was removed from the tube and finely macerated (by means of the jagged end of a tube of Pyrex glass) on a piece of tested filter paper. The minced *Laminaria* was put back into the tube and rinsed 6–10 times with sea water until none of the liberated pigment could be distinguished in the sea water. Fresh sea water was then added and the respiration determined. The results are given in table VIII, the control data being given in table V. In table VIII it will be observed that the relative amount and relative rate of respiration are both

TABLE VI A

CHANGE IN PH VALUE OF SEA WATER PRODUCED BY RESPIRATION OF *Laminaria* DURING 3 PERIODS (23 MIN. EACH) IN SEA WATER; AT END OF SECOND PERIOD MATERIAL PLACED IN RUNNING TAP WATER FOR 19 HOURS; MATERIAL THEN PLACED IN SEA WATER AT 22° C. FOR 34 MINUTES BEFORE BEGINNING THIRD PERIOD

Period	Change in PH	Change in PH after exposure to tap water = A	Change in PH before exposure to tap water, calculated from first 2 periods = B	Relative amount of respiration = A/B	Time in min.	Time (in min.) of exposure after exposure to tap water	Relative rate of respiration
1.....	8.0−7.7=0.3	.....	.....	.....	23	0	.....
2.....	8.0−7.7=0.3	.....	.....	.....	46	0	.....
3.....	8.0−8.0=0.0	0	0.3	0	69	23	0.0÷0.3=0.0

TABLE VII

CONTROL FOR VII A: 4 PERIODS (25.5 MIN. EACH) IN SEA WATER; BETWEEN SECOND AND THIRD PERIODS MATERIAL KEPT IN SEA WATER AT 16° C. FOR 70 MINUTES

Period	Change in PH	Total change in PH = A	Change in PH calculated from first 2 periods = B	Relative amount of respiration = A/B	Time in min.	Relative rate of respiration
1.....	8.0−7.57=0.43	0.43	0.425	1.01	25.5	0.43÷0.425=1.01
2.....	8.0−7.58=0.42	0.85	0.85	1.00	51.0	0.42÷0.425=0.99
3.....	8.0−7.58=0.42	1.27	1.275	1.00	76.5	0.42÷0.425=0.99
4.....	8.0−7.58=0.42	1.69	1.70	0.99	102.0	0.42÷0.425=0.99

TABLE VII A

CHANGE IN PH VALUE OF SEA WATER PRODUCED BY RESPIRATION OF *Laminaria* DURING 4 PERIODS (28 MIN. EACH) IN SEA WATER; BETWEEN SECOND AND THIRD PERIODS MATERIAL PLACED IN LARGE VOLUME OF SEA WATER AND KEPT AT 35° C. FOR 70 MINUTES

Period	Change in PH	Change in PH after exposure following second period = A	Change in PH immediately after second period = B	Relative amount of respiration = A/B	Time in min.	Time (in min.) of exposure during last two periods	Relative rate of respiration
1.....	8.0−7.25=0.75	.....	.....	.....	28	.....	.....
2.....	8.0−7.28=0.72	.....	.....	.....	56	.....	.....
3.....	8.0−7.80=0.20	0.20	0.735	0.27	84.	28	0.20÷0.75=0.26
4.....	8.0−7.85=0.15	0.35	1.470	0.24	112	56	0.15÷0.75=0.20

more than doubled, but gradually decline. After 1 hour the relative rate of respiration was still above the normal. In this case the time of death could not be determined.

The experiments show that although the rate of respiration may be maintained for a time after death, it gradually falls off and eventually becomes very small. The question arises whether this falling off is due to exhaustion of the supply of oxidizable material or not. It is clear that when respiration has practically ceased there is a considerable amount of organic material left, but it is by no means certain that this material is such as to be easily oxidized by the ordinary processes which produce CO_2. On the

TABLE VIII

CHANGE IN PH VALUE OF SEA WATER PRODUCED BY RESPIRATION OF *Laminaria* DURING 5 PERIODS (31.25 MIN. EACH) IN SEA WATER; BETWEEN SECOND AND THIRD PERIODS MATERIAL FINELY MINCED

Period	Change in PH	Change in PH after mincing = A	Change in PH before mincing, calculated from first 2 periods = B	Relative amount of respiration = A/B	Time in min.	Time (in min.) of exposure after mincing	Relative rate of respiration
1.....	8.1−7.70=0.40	.....	.....	.....	31.25	.....	.....
2.....	8.1−7.70=0.40	.....	.....	.....	62.50	.....	.....
3.....	8.1−7.05=1.05	1.05	0.40	2.6	93.75	31.25	1.05÷0.40=2.6
4.....	8.1−7.65=0.45	1.50	0.80	1.9	125.00	62.50	0.45÷0.40=1.1
5.....	8.1−7.90=0.20	1.70	1.20	1.4	156.25	93.75	0.20÷0.40=0.5

other hand, we must consider the possibility that the production of CO_2 falls off because the supply of oxidizing enzymes is used up. Various observers have found that these enzymes may be used up (or inactivated) during oxidation (**1, 9**). If the process of oxidation involves the cooperation (or successive action) of various enzymes the inactivation of any one of them might bring the whole process to a standstill.

WARBURG (**21**), as the result of extensive study, has come to the conclusion that the rate of oxidation depends on the amount of "structure" which the cell possesses. If the "structure" is partially or completely destroyed the oxidation diminishes in propor-

tion, except in rare cases (as in the unfertilized sea urchin egg) where the oxidation is independent of structure. He states that the latter case disposes of the "reaction chamber" theory of cell structure, according to which the substances necessary for oxidation are separated by the semipermeable membranes of the cell in such a way as to regulate the speed of oxidation, for these substances can be completely mixed, as in the cytolysis of the unfertilized sea urchin egg, without any change in the rate of oxidation.

WARBURG's treatment of the "reaction chamber" hypothesis seems to rest upon a misunderstanding. It is quite possible that in the cytolysis of the sea urchin egg the "reaction chambers" are not destroyed, since each of the fine granules into which the egg is resolved by cytolysis may be such a "reaction chamber" surrounded by a semipermeable surface.[4] In case some or all of the reaction chambers are destroyed by the treatment, because they are larger or for any reason more sensitive to the treatment, a change in the rate of oxidation may be expected (either an increase or decrease, according to circumstances). WARBURG himself states that where an increase of chemical action results from the injury the "reaction chamber" hypothesis seems to be justified. This is precisely what the writer finds. Increase of oxidation as the result of injury (although not as the result of death) has previously been recorded by many observers (5).

The "reaction chamber" hypothesis has much in its favor. An especially good example is the bitter almond, which at once produces HCN upon injury. In this case the reacting substances are known and we cannot escape the conclusion that previous to injury they fail to react because they are kept apart by structures in the cell. In some cases the mingling of substances, owing to the breaking down of such separating structures, can distinctly be seen under the microscope. This is the case with the marine alga *Griffithsia*, as described by OSTERHOUT (14, 15). When cells of this alga are injured by poisons (NH_4Cl), or mechanically, or by cytolysis with dilute sea water, the chromatophores (which contain a soluble red pigment) become permeable and the pigment can be seen passing

[4] The existence of an actual membrane is unnecessary.

out into the surrounding cytoplasm. It would seem, therefore, that in the absence of a better explanation[5] the reaction chamber hypothesis might serve a useful purpose.

Summary

The respiration of *Laminaria* after death may be considerably greater than in its normal condition. This is the case when it is killed by alcohol, acetone, formaldehyde, and ethyl bromide, as well as by drying and by other methods.

LABORATORY OF PLANT PHYSIOLOGY
HARVARD UNIVERSITY

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[5] The explanation suggested by WARBURG (21), that the oxidizing substances are bound up by the structure, seems too vague for discussion.

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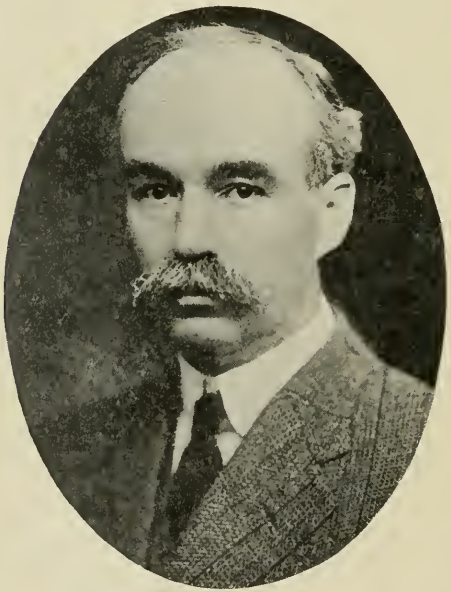
BRIEFER ARTICLES

GEORGE FRANCIS ATKINSON

(WITH PORTRAIT)

In the death of GEORGE FRANCIS ATKINSON on November 14, 1918, America lost one of her great botanists. Born in the little village of Raisinville, Monroe County, Michigan, on January 26, 1854, he received his preliminary collegiate training in Olivet College in that state. From there he went to Cornell University, where he took the degree of Ph.B. in 1885. Immediately upon graduation he became assistant professor of general zoölogy at the University of North Carolina. The following year he was made associate professor, remaining there until 1888, when he was called to a full professorship in botany and zoölogy in the University of South Carolina. In 1889 he was appointed professor of biology and botany in the Alabama Polytechnic Institute, where he remained until 1892. In 1892 he was called to Cornell University as assistant professor of botany, became associate professor in 1893, and upon the death of Professor Prentiss in 1896 was made full professor and head of the department. He was also for many years the botanist of the Cornell Agricultural Experiment Station. He continued head of the Department of Botany in the Arts College in Cornell University until his death.



Upon the request of an organization of his former students, the Board of Trustees of the University in 1917 relieved him of all teaching

and administrative burdens in order that he might devote his entire time and energies to the completion of his monographic studies on the fleshy fungi of North America.

In the vigorous and enthusiastic pursuit of this enterprise he made an extensive collecting trip through the Atlantic seaboard states from Florida to the District of Columbia in the spring and summer of 1918. Returning to Ithaca in September he left after an all too short rest for the Pacific Coast, there to pursue his studies of the fleshy fungus flora of that region. On this trip he was without any assistant and most of the time alone. A former student, Dr. ADELINE AMES, spent a few days collecting with him in the region about Tacoma, Washington, shortly before his death.

Urged by the wonderful variety and abundance of the forms he found and an indomitable enthusiasm for his work, he apparently labored beyond his strength and exposed himself to unusual hardships. He took a heavy cold from exposure on a trip into the mountains near Tacoma, Washington, which rapidly developed into influenza followed by pneumonia. He died in the City Hospital at Tacoma far from friends and kindred, another martyr to the cause of botanical science.

Professor FRYE of the University of Washington upon news of his death went immediately to Tacoma to learn the details and to rescue his notes and collections. Dr. AMES also went again to Tacoma shortly thereafter. Thanks to their generous and painstaking efforts we have a full account of Professor ATKINSON'S last days. This record gives us a wonderful insight into the man's devotion to his work and a fuller appreciation of his greatness.

Interested primarily in entomology in the early days of his career, he soon turned to the botanical field, and especially mycology, in which perhaps he has made his most notable investigations. He was without doubt the greatest American student of the fleshy Basidiomycetes. His numerous contributions in this field and a remarkably large and exceptionally excellent collection of photographs, together with specimens and notes on these forms not only American but European attest his preeminence in this field. He was, however, a botanist of wide interests, and his investigations and writings touch nearly every branch of this broad field. A true philosopher, he gave to his contributions that philosophical character and flavor which is the mark of scientific genius.

He was the author of many textbooks, notable among which are several elementary and college textbooks of general botany, "The biology of ferns," and "Mushrooms, edible, poisonous, etc." He made many

contributions to the botanical journals, not only of America but also of England, France, and Germany.

His travels in Europe, his extensive correspondence, and the students that came from the ends of the earth to study in his laboratories have made his name familiar in the botanical institutions of every land. As a delegate to the International Botanical Congresses of 1905 and 1910 held in Vienna and Brussels respectively, he made for conservatism in botanical nomenclature. A charter member of the Botanical Society of America and at one time president, he has been for a generation one of the leaders of American botanical thought and activity. He was a fellow of the American Association for the Advancement of Science, a member of the American Philosophical Society, and in 1918 was elected to the National Academy of Science. He was for years an associate editor of the BOTANICAL GAZETTE. He was also a member of the honorary societies of Phi Beta Kappa and Sigma Xi.

To those of us who knew him intimately as teacher and friend, our days with him in field and laboratory will ever remain a happy and a grateful memory. He was a master of the highest scientific ideals, unsparing in his criticisms, just and fair in his judgments, generous with help and suggestions, a good friend and a genial companion.—H. H. WHETZEL, *Cornell University, Ithaca, N.Y.*

CURRENT LITERATURE

BOOK REVIEWS

Manual of tree diseases[1]

This first wholly American work in book form on the diseases of forest trees is one of "The Rural Manuals" edited by L. H. Bailey, and in conformity with the general plan of this series has been written primarily for the general public. Insect and other animal injuries are not included. The treatment of the subject throughout is simple and direct; the diseases are concisely described, and methods of control indicated. The first 4 chapters deal with such maladies of biotic and abiotic origin as are common to many kinds of trees and are respectively entitled "Seedling diseases and injuries," "Leaf diseases and injuries," "Body and branch diseases and injuries," and "Root diseases and injuries." Chapters v–xxxii are devoted to an account of the more "specific" diseases, one chapter to each generic host group, beginning with the alders. The arrangement of the chapters is alphabetical according to the English host group names. Two chapters follow, one on "Tree surgery," the other on "Spraying and dusting for leaf diseases." The book is equipped with a glossary, a general bibliography of tree diseases, and an excellent index.

This work, although not intended as a textbook, will be welcomed by all students of plant pathology because it is the only summary available of the diseases of the forest trees of the United States and of Canada, and because it includes many classified references to the literature. The writing of the book reveals the limitations of forest pathology in America; the number of workers in this field has been small, the subject matter is as yet largely unexplored, and the applications of the results so far attained have been restricted. The author clearly recognizes these facts, and does not fail to point out the direction investigations should follow; in so doing he makes a contribution of prime importance.—J. H. Faull.

MINOR NOTICES

Our national forests.—The period of reconstruction not less than the progress of the war has directed, in a special manner, the attention of our people to their natural resources and to the desirability of properly utilizing and conserving them. Thus no more timely moment could be chosen for the publication of some account of our forest wealth as shown in the establishment and

[1] Rankin, W. Howard, Manual of tree diseases. pp. 398. *figs.* 70. 1918. New York: Macmillan Co.

management of our national forests. BOERKER[2] has collected and organized a mass of scattered data and presented them in a very readable form. While particularly well suited in its style of presentation to appeal to the general public, it will prove equally welcome to foresters and botanists who wish to know the history of the organization of these forests and the different forms of administration under which they have attained their present dimensions. The addition of a bibliography would have added much to the scientific value of the volume without detracting from its popular interest. It may also be criticized because of the lack of a suitable index to facilitate reference; but on the whole the work is well done, the material has been well organized, is attractively presented, and so far as the reviewer is able to judge the data are entirely accurate and reliable.—GEO. D. FULLER.

Grasses and grasslands of South Africa.—In order to facilitate the study of the extensive grasslands of South Africa, BEWS[3] has prepared a series of keys for the identification of the 500 species of grasses which form so conspicuous a portion of the flora of that part of the continent. These keys seem to be well suited to serve the purpose for which they are intended, but the other parts of the volume are of far more interest to the American reader. In them are discussed: (1) the structural and ecological characteristics of the principal species; (2) general character of the grasslands and the development of the various association types; and (3) economic application of the ecological principles involved. It is interesting to find types comparable to the "short grass," "wire grass," and "prairie grass" of North America, as well as a tall coarse *Andropogon* association, this last developing upon potential woodland areas, and a mountain tussock grassland. The discussion of the successional relations of these and other association types into which grasses enter gives a comprehensive general sketch of the plant communities of the major portion of South Africa.

In the final chapter the feeding value of the different types of grassland, as well as the comparative merits of native and introduced species, is discussed. The effect upon the productivity of various types of grassland by various kinds of grazing and the results from grass burning are considered and some of the ecological problems involved are pointed out. An appendix contains a list of English, Dutch, Zulu, and Sesuto names of the more important species.—GEO. D. FULLER.

NOTES FOR STUDENTS

Vegetation of Cape Breton.—Separated from the mainland of Nova Scotia by a narrow strait, the island of Cape Breton lies between the Gulf of St. Lawrence and the Atlantic in latitude 45–47° N. It possesses a climate

[2] BOERKER, RICHARD, H.D., Our national forests. pp. lxix+238. *figs.* 80. 1918. New York: Macmillan Co.

[3] BEWS, J. W., The grasses and grasslands of South Africa. 8vo. pp. 161. *figs.* 24. *map.* Pietermaritzburg: Davis & Sons. $2.00 (postpaid from author).

characterized by long winters and short cool summers, the extremes of temperature being modified by the close proximity of the ocean. A rainfall of 50 in. per year and frequency of fogs make the water supply sufficient for a luxuriant vegetation, which has been carefully studied by NICHOLS.[4] He finds two climatic forest formations represented, the deciduous type upon the lowlands which fringe the coast, and the coniferous type upon the granite uplands which occupy the entire interior portion of the island. These are about 1000 ft. above sea level and form a slightly undulating glaciated surface.

The lowlands show many associations depending upon the stage of development attained, and these variations and the successive stages which have led to their development are carefully discussed and the climax shown to be a forest dominated by beech, sugar maple, and hemlock, together with small quantities of *Betula lutea*, *Picea canadensis*, and *Abies balsamea*. The abundant details of these studies cannot be noticed in a brief review, but two problems in the relationship between the deciduous and evergreen elements of the vegetation are decidedly interesting. It has been found that upon the destruction of the deciduous forest by culling or burning it is succeeded by a coniferous stand dominated by *Abies balsama* and *Picea canadensis;* and further that the climax deciduous forest possesses a very considerable percentage of small *Abies balsamea* which never seem to succeed in competition with the other tree members of the association. NICHOLS presents evidence showing that the balsam fir is fairly shade tolerant, and that its lack of success is due to its short life, maturity being attained in about one century, and to its great susceptibility to fungus diseases.

It seems evident that the coniferous forest dominated by *Abies* and *Picea* is the climatic rather than the edaphic climax of all portions of the island exceeding 700 ft. in elevation. The factors which appear to differentiate the climate of the uplands from that of the lowlands are the greater extremes of temperature and the greater humidity due to fogs and low-hung clouds which frequently envelop the more elevated areas.

This upland forest is of decided importance in the production of pulp wood, its contents being estimated at 12,000,000 cords. Upon the more exposed parts of the uplands are developed "the barrens," closely resembling the tundras of the subarctic. The low vegetation of "the barrens" varies from a degenerate coniferous forest of the Krummholz type, where the distorted trees are limited in height to the thickness of the snow cover, to coniferous and ericaceous heaths, and to bogs of varied character. These bogs occupy considerable portions both of the lowlands and "the barrens," their most striking form being the raised peat bogs of the latter region, which have received careful attention, so that many problems connected with their development have been elucidated.

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[4] NICHOLS, GEO. E., The vegetation of northern Cape Breton Island, Nova Scotia. Trans. Conn. Acad. 22:249–267. *figs.* 70. 1918.

NICHOLS finds the bogs of the raised type, corresponding to the "Hochmoors" of Europe, occurring upon this continent in Newfoundland and those parts of eastern Canada and Maine which are in close proximity with the sea coast. The climatic factors necessary for their development are abundant precipitation, relatively low atmospheric humidity, cool summers, and the absence of extremely low temperatures such as prevail farther inland. One of the necessary edaphic factors is an impervious substratum, here furnished by the Laurentian rocks. This is vitally important, since the source of water supply is the rainfall and not springs, as some have assumed.

The early stages of these raised bogs are not essentially different from those obtaining in bogs of the more common and familiar type, but their subsequent development is dependent upon the presence of distinct types of *Sphagnum*. NICHOLS classifies these mosses into 5 ecological groups, beginning with the decidedly aquatic and ending with those of comparatively xerophytic habits. It is upon the growth of the mesophytic and xerophytic sphagnums that the development of the dry raised bog depends. These mosses are cushion-forming in habit, and their successive development elevates the central portions of the bog many feet above its rim. Such a raised bog presents a hummocky surface, and except in wet weather a rather firm springy substratum quite dry underfoot. Upon the surface in addition to the xerophytic *Sphagnum* are other mosses such as *Racomitrium* and *Polytrichum*, some fruticose lichens, and several ericaceous shrubs for the most part less than a foot high. Scattered and dwarfed specimens of *Larix* and *Picea mariana* also occur. A typical specimen of the former, scarcely a foot high, possessed a trunk 1 inch in diameter showing more than 50 annual rings.

Another striking feature of the region seems to be the development of subsequent ponds within the bog area. These differ decidedly from the marginal trenches described by STALLARD,[5] which are due to fire consuming the peat in the shallow marginal portions of the bog during periods of unusual drought. The ponds in the Cape Breton bogs are due to the impervious nature of the peat from some of the sphagnums forming barriers and dams which obstruct the drainage on gentle slopes. Such ponds function as storage reservoirs, retaining much of the water which accumulates in them during wet periods and thus insuring to adjacent areas a constant supply throughout the season.

The development of the raised bogs, the subsequent bog ponds, and other features of the vegetation are illustrated by diagrams and photographs. The various successions are carefully traced and clearly described, the various communities being classified according to the system already noted.[6] In its comprehensive character, its abundance of detail, and its notable contributions to various phases of ecology, including the relationships between deciduous and coniferous forests, the ecology of the sphagnums and of the development of

[5] STALLARD, HARVY, The origin of *Sphagnum* atolls. New Phytol. 15:250–256. 1918.

[6] BOT. GAZ. 66:385–388. 1918.

raised bogs, this report stands as one of the most notable of recent years.—Geo. D. Fuller.

A new fixative for paraffin sections.—Dr. Koloman Szombathy[7] describes a new method of fixing paraffin sections to the slide. The fixative is claimed to have the advantage of not being dissolved by alkaline stains, and furthermore in not being stained by hematins and aniline stains such as eosin fuchsin, orange G., etc. The formula given by him is as follows: gelatin 1 gm., distilled water 100 cc., salicylate of soda (a 2 per cent solution) 1 cc., pure glycerine 15 cc.

Dissolve the gelatin in water at 30°, add the salicylate of soda, shake well, cool, and filter. To this add 15 gm. of pure glycerine. The solution obtained should be perfectly clear. A small amount of the fixative together with a drop or two of a 2 per cent formalin solution is placed on the slide, smeared evenly over the surface, and rubbed in well. Care should be observed that the formalin is mixed with the fixative. The sections or paraffin ribbons are then placed on the fixative and permitted to dry in the thermostat or any other warm place which is protected from dust. The formalin "tans" the gelatin and makes it insoluble. A modification of the method consists in exposing the slides, which have been mounted without the use of formalin solution, to vapors of concentrated formalin in a thermostat. The effect of the formalin is identical. A third method consists in preparing a solution of equal parts of 1 per cent gelatin in water and 2 per cent formalin. The fixative is then used as recommended for albumen fixative.

The writer has tested the fixative recommended by Szombathy and finds it to be an excellent one. Material known to be difficult to retain on the slide was tried out. Sections of grass leaves and moss archegonia adhered to the slide even when the latter were left in running water for several days or exposed to a strong solution of hydrogen peroxide. Alkaline stains do not dissolve the gelatin nor do the stains tested stain the background to an appreciable extent.

Of the 3 methods originally recommended, the following modification gives the most satisfactory results. Make up the fixative according to the first formula, put a drop on the slide, and smear it evenly over the surface. Float the paraffin ribbon on the slide on a 2 per cent formalin solution. Warm the slide gently on the usual copper plate and, after the ribbon has straightened and become smooth, drain off the surplus water and let the preparation dry. When one is dealing with material which does not stick to the slide easily, it will be found of advantage to put a small dish of formalin in the thermostat where the preparations are drying, since the formalin vapors help in rendering the gelatin insoluble.

This new fixative is very easily prepared, keeps well, and does hold the sections to the slide. It should come into general use especially for material which does not adhere to the slide under ordinary conditions and when stains

[7] Szombathy, Koloman, Neue Methode zum Aufkleben von Paraffinschmitten. Zeitschr. Wiss. Mikr. **34**:334–336. 1918.

are employed which are alkaline in nature and have the objection of staining the background.—ERNEST F. ARTSCHWAGER.

Size variation in secondary xylem.—BAILEY and TUPPER[8] have applied the comparative method in thoroughgoing fashion to an attack on the problem of cell size. Confining themselves to a study of the length of the tracheary elements in the secondary xylem of trees and shrubs among vascular cryptogams, gymnosperms, and angiosperms, they present data derived from thousands of measurements on some 440 species belonging to 124 families. The most conspicuous fact brought out by this reconnaissance survey is that the length of these elements is roughly correlated with phylogenetic position, being greatest in vascular cryptogams, somewhat less in gymnosperms, and least in angiosperms. This progressive reduction in the length of the wood cells has been associated with the reduction in amount of the primary xylem in the passage from lower to higher forms, but is probably due in greatest measure to the evolution and differentiation of vessels. These elements have become progressively shorter and broader, thus losing their resemblance to the primitive tracheid; and the fibers and tracheids associated with them have also grown shorter, although naturally to a less extent. Notable exceptions to the general rule are the vessel-less Magnoliaceae and Trochodendraceae, represented by *Drimys* and *Trochodendron*, which possess tracheary elements far longer than other angiosperms, and thus resemble the gymnosperms. Evidence from this source obviously supports the view that these genera are primitive rather than reduced types.

The authors have also made a preliminary study of the relations between the length of the tracheary elements and the age of the plant, its growth habit, and the environment under which it lives. So far as the cells studied are concerned, there is no definite correlation between body size and cell size. The tracheary elements may increase in length for a few years as the plant grows larger, but they soon reach a constant size. Dwarfed and depauperate plants tend to have somewhat smaller elements than normal individuals.

The authors point to the need of more intensive investigations in this hitherto almost unexplored field; and in particular call for a careful study of the activities of the cambium and the factors which direct these activities. Indeed the growing point of plants, once so enthusiastically studied as the key to histology and then for so long neglected, bids fair to be once more a center of interest as one of the keys to a knowledge of morphogenesis.—E. W. SINNOTT.

Sap concentration in epiphytes.—Continuing the studies already noted[9] upon the concentration of tissue fluids, HARRIS[10] has found in several species of

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[8] BAILEY, I. W., and TUPPER, W. W., Size variation in tracheary cells. I. A comparison between the secondary xylems of vascular cryptogams, gymnosperms, and angiosperms. Proc. Amer. Acad. 54:149–204. *figs. 6.* 1918.

[9] BOT. GAZ. 65:285–286. 1918.

[10] HARRIS, J. ARTHUR, On the osmotic concentration of the tissue fluids of desert Loranthaceae. Mem. Torr. Bot. Club 17:307–315. 1918.

Phoradendron growing in the Arizona deserts upon various hosts, such as species of *Acacia*, *Quercus*, *Fraxinus*, and *Populus*, that the osmotic concentration of the tissue fluids of the parasite is generally greater than that of the host. The concentration of the fluids of such parasites in this semidesert region is also greater than and usually about twice as great as that of similar plants found in the mountain rain-forests of Jamaica. These results quite agree with our expectations, but in a further paper the same investigator[11] clearly demonstrates the errors that would be involved in generalizing broadly on insufficient data.

The later investigations have to do with the tissue fluids of epiphytic Bromeliaceae, Orchidaceae, Piperaceae, and Gesneraceae, and these are shown to possess a decidedly lower concentration than those from terrestrial vegetation. In the mountain rain-forests of Jamaica the epiphytes show 37–60 per cent of the concentration commonly found in herbaceous terrestrial vegetation and 28–45 per cent of the concentration characteristic of ligneous soil plants. The epiphytes of the Jamaican rain-forests show lower concentrations than related plants of the same habit growing in the subtropical forests of Florida. The exactness of the data and quantitative character of the comparisons make these investigations important, and lead us to look forward for the further results promised in the study of parasitism by quantitative methods.—Geo. D. Fuller.

Bennettitales.—Two cones of the Bennettitales from the British Cretaceous, one of them a new species, have just been described by Stopes.[12] The first and most important is the one upon which she has founded the new species *B. albianus*, the specific name referring to the strata in which the specimen was found. Only a small piece of a single cone was found, but it was very well preserved. After a study of the topography, the entire fragment was cut, yielding 2 longitudinal and 5 transverse sections, the latter passing through the seeds and the former through their stalks. The most striking feature of the cone is its large size, not less than 70 mm. in diameter and probably more. The seeds are innumerable, as many as 600 showing in a single transverse section of the fragment. The seeds are 5–6 mm. long and 1.2 mm. in diameter, thus contrasting with the more or less ovoid seeds already described. The interseminal scales are fused around the apex of the seed. The embryo has 2 cotyledons and a rather massive hypocotyl and radicle.

The other specimen, *B. maximus*, was described from superficial characters by Carruthers in 1870. The present study shows that the vascular axis is very small for such a large plant and the cones are bisporangiate, the first petrified bisporangiate cones which have been found in England. The cones

[11] Harris J. Arthur, On the osmotic concentration of the tissue fluids of phanerogamic epiphytes. Amer. Jour. Bot. 5:490–506. 1918.

[12] Stopes, Marie C., New Bennettitean cones from the British Cretaceous. Phil. Trans. Roy. Soc. London **208**:389–440. *pls. 19–24*. 1918.

are very young but do not seem to have been well preserved. If material in this stage and somewhat older stages could be secured, it would help immensely in comparing the Bennettitales and the Cycadales.—C. J. CHAMBERLAIN.

Cytology of the basidium.—A cytological investigation of the basidium of *Eocronartium muscicola*, one of the Auriculariales parasitic upon mosses, was undertaken by FITZPATRICK[13] because he had noticed that the nuclei are of unusual size, and because very little cytological work has been done in this order. The mycelium, which is intracellular and extends throughout the host, is composed of binucleate cells. The cells of the sporophore are also binucleate, and, during division, it is seen that the number of chromosomes in each of the 2 nuclei is 4. During the development of the basidium, the 2 nuclei fuse, the resulting nucleus passes into synapsis, and in later stages of division shows 4 chromosomes, which is also the number at the second division, so that the total number of chromosomes in the cell is reduced. Toward the close of the second division a transverse wall appears in the middle of the basidium and is soon followed by two more walls, so that the basidium consists of a filament of 4 cells. The sterigmata, which are large in proportion to the cells from which they arise, are not quite simultaneous in their appearance. The chromatin becomes drawn out into a slender thread as the nuclei pass into the young spores, and there is no connection with the centrosomes, as has been reported for some basidia. How the binucleate mycelium arises from the uninucleate spore has not yet been determined.—C. J. CHAMBERLAIN.

Orientation of roots.—HOLMAN[14] has investigated the influence of the medium upon the orientation of primary terrestrial roots. He shows that the failure of roots grown in air to reach a vertical position is due to lack of mechanical resistance to the advance of the root tip after the flattening of the primary geotropic curvature, rather than to differences in water content in the medium, or changes in geotropic sensitiveness, or to thigmotropism. His observations have been extended to secondary roots,[15] and here also he finds that when they have been displaced from normal position with respect to gravity, and the first curvature of response has been flattened, mechanical resistance is necessary to a complete reaction to normal position. The mechanical resistance hinders flattening of the primary curvature of the root tip, and passively depresses the tip as it moves forward, thus reinforcing and completing the geotropic response. —C. A. SHULL.

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[13] FITZPATRICK, H. M., The cytology of *Eocronartium muscicola*. Amer. Jour. Bot. 5:397–419. *pls. 30–32*. 1918.

[14] HOLMAN, RICHARD M., The orientation of primary terrestrial roots with particular reference to the medium in which they are grown. Amer. Jour. Bot. 3:274–318. 1916.

[15] ———, Influence of the medium upon the orientation of secondary terrestrial roots. Amer. Jour. Bot. 3:407–414. 1916.

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EDITED BY

JOHN M. COULTER

With the assistance of other members of the botanical staff of the University of Chicago

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THE
BOTANICAL GAZETTE

MAY 1919

EFFECT OF ANESTHETICS UPON RESPIRATION

A. R. C. HAAS

(WITH SEVEN FIGURES)

The special interest which this subject has acquired, as the result of certain modern theories, makes it desirable to give a brief review of some of the more important contributions (5, 10) to our knowledge of it.

MEYER (19) and OVERTON (21) independently concluded that the effect of a narcotic increases with its solubility in substances of a lipoid nature. According to them narcosis does not appear until the lipoids of the cells have absorbed the narcotic to a definite molecular concentration (21). The theory has been criticized (5, 10) because it fails to explain why narcotics, such as benzamide and monacetin, which at higher temperature are less soluble in fat, have an effect which increases with the temperature. Both MEYER and OVERTON recognize the fact that often there is no relation between the narcotic power of a substance and its relative solubility in oil; the partition quotient for isobutyl alcohol is about 180 times greater than for ethyl alcohol, but its power to cause narcosis is only about 6 times as great as that of ethyl alcohol. They state that such cases cannot be used as arguments against the lipoid theory in case the narcotic is not chemically indifferent, but has a special reaction affinity, as is the case, for example, with the basic narcotics.

VERWORN (29) has advocated the view that narcotics interfere with the oxygen carriers of the cell and render them incapable of

activating the molecular oxygen. As a result oxidation cannot take place and disintegration occurs, the cells thereby being asphyxiated. In this connection he makes the statement that a more or less complete recovery from narcosis, which may occur even in an oxygen-free medium, is at the cost of the oxygen contained within the living substance, which (on account of the suppression of the oxidation processes) could not be consumed.

MANSFELD'S (16) view is not essentially different from that of VERWORN. He believes that because the lipoids take up the narcotic, their power to absorb oxygen is decreased. Narcotized cells cannot take up sufficient oxygen for their needs and hence irritability is decreased by lack of oxygen. That narcotics do decrease the ability of olive oil to dissolve oxygen, has been asserted by HAMBURGER (8), although objection to his experiments has been made by WINTERSTEIN (31). In many cases it is certain that narcosis has nothing to do with absorption of oxygen. Thus WINTERSTEIN has observed that on anesthetizing the anaerobic worm *Ascaris* in absence of oxygen, it comes to rest very quickly under the influence of the anesthetic.

Experiments by numerous investigators have shown that narcosis and decrease of oxidation are not parallel. WARBURG (30) has observed that some narcotics decrease the oxidation of the erythrocytes of geese as much as 30–70 per cent. He has also observed, however, that narcotics do not always decrease the consumption of oxygen, for he has found that the segmentation of fertilized sea urchin eggs can be inhibited by phenylurethane without a perceptible decrease of the consumption of oxygen. LOEB and WASTENEYS (14) have obtained similar results with chloral hydrate, chloroform, and alcohol. NOTHMANN-ZUCKERKANDL (20) observed that the protoplasmic streaming of plant cells is quickly brought to a standstill by narcotics. Such cells have been shown to be rather insensitive to lack of oxygen, in that the streaming is stopped in the absence of oxygen only after several weeks.

BÜRKER (4) was of the opinion that because of the great solubility of narcotics in the lipoid of the cells, there is a competition between the lipoids and other substances in the protoplasm for the active oxygen, such that the cells are more or less in a state of

asphyxiation. His hypothesis maintains that the absorption of oxygen is not reduced during narcosis, but that the oxygen is prevented from going to its usual point of attack. This would necessitate the assumption that oxygen is more readily absorbed by the lipoids than by the other substances of the cell, which is not the case. An objection to all theories which make narcosis conditional on lipoid solubility is the fact that magnesium sulphate and carbon dioxide, which are not soluble in lipoid, produce typical anesthesia.

HÖBER (10) has formulated the hypothesis that narcosis is due to inhibition of enzymatic processes, brought about by a decrease in dispersion. This hypothesis is favored by the investigations of BATELLI and STERN (2), who found that proteins, such as the nucleo-proteins, are influenced by narcotics in approximately the same relative and absolute concentrations as those at which the enzymes are affected. In this connection VERNON (28) found that most narcotics are harmless to oxidases up to a definite limiting concentration, beyond which injury occurs. HÖBER explains the retardation or inhibition of enzyme action as due to the fact that narcotics go into the surface between the enzymes and the medium in which they are dispersed, thereby displacing the substratum on which the enzymes act.

The investigations hitherto discussed are largely based on measurements of the absorption of oxygen. In many cases such measurements could be made more accurately than determinations of the amount of carbon dioxide produced. Since the writer has recently been able to develop a method for the measurement of minute amounts of carbon dioxide in solution, it seemed to him that a fresh investigation of the subject by means of this method was desirable.

Previous investigations on the effect of narcotics on the production of carbon dioxide have yielded somewhat contradictory results. APPLEMAN (1) found that vapor of ethyl bromide approximately doubles the respiration of potatoes. MAYER (18) found that 0.25 per cent prussic acid stops the respiration of higher plants entirely. SCHROEDER (24) observed a decrease in respiration when *Aspergillus* was treated with ether. He found that prussic acid

inhibited the production of CO_2, while the absorption of oxygen continued. KOSINSKI (12) found that low concentrations of ether increased the respiration of *Aspergillus*, while higher concentrations decreased it. LAURÉN (13) and also IRVING (11) have noted that respiration increases during anesthesia produced by ether and chloroform. TASHIRO (26) found that anesthetics greatly reduce the output of CO_2 by dry seeds. BONNIER and MANGIN (3), as the result of experimental work upon the influence of measured quantities of ether upon flowering plants, concluded that the respiratory activity is unaffected by anesthetics. It has since been shown by EWART (6) that chloroform increases the respiratory activity in *Elodea*.

The effect of anesthetics upon the respiration of marine plants has received very little attention. HARDER (9) has made determinations of the respiration of marine algae, but in no case has he studied the effect of anesthetics. PANTANELLI (22) has observed that sea water, when half saturated with chloroform, reduces the excretion of CO_2 to about one-half of the normal. His experiments were very few, and no duplicate or control experiments were made. The methods employed by PANTANELLI and HARDER often required that plants be shut up air-tight, in flasks completely filled with sea water, and left in this condition for several hours, analyses being made at the beginning and end of these long periods.

In the experiments of the writer on the effect of anesthetics upon the production of CO_2, the marine alga *Laminaria* was found to be well suited to the purpose. Fronds were cut up into pieces about 2 inches long. Each piece was rolled up loosely and inserted into a piece of Pyrex glass tubing. This was closed at one end, while a piece of paraffined rubber tubing was attached to the open end. Sea water, of approximately the same temperature as the material, was then added to the tubes and the rubber tube closed by a spring clamp. The tubes were then brought very gradually to the temperature (16° C.) of the constant temperature bath. The tubes were kept dark by inserting each tube in a black-enameled, collapsible tin tube submerged in the bath. Several tubes were used as controls in each experiment. These contained material in sea water without any addition. The reagents were

always used at the same temperature as that of the water bath. After the tubes containing the *Laminaria* in sea water had been at 16° C. from half an hour to an hour, the solution was poured out of each tube and replaced by fresh sea water at 16° C. This was repeated several times before beginning an experiment.

After a tube containing *Laminaria* in sea water had been clamped off so as to include a small bubble of air which could be used as a stirrer, and had been exposed to 16° C. for a definite period, it was removed from the bath. The solution was slightly stirred by inverting the tube a few times; the clamp was then opened and the solution rapidly poured into another empty tube to which the same number of drops of indicator (phenolsulphonephthalein) had been added as was added to the buffer solutions (7). In order to mix the solution with the indicator it was stirred as described, and was then compared with buffer solutions of a known PH value which contained the same amount of indicator.

The use of a small bubble of air as a stirring agent was found to be very convenient, and when compared with the use of paraffined glass globules as stirrers was found to introduce no error of any importance.

The buffer solutions (27) were made up by mixing M/15 Na_2HPO_4 and M/15 KH_2PO_4 in various proportions. They were of the same diameter as those containing the alga. A 0.01 per cent aqueous solution of phenolsulphonephthalein served as the indicator of the PH values. The indicator solution was used at the rate of 5 drops in 10 cc. of solution. A correction (30) of 0.30 for the salt error of the indicator in sea water was subtracted from the observed readings of the PH value of sea water. The use of a constant source of light (the "Daylight" lamp) permitted observations to be made in a uniform manner. The decrease in PH, which results from the production of CO_2, served as a criterion of the amount of respiration.

In all the experiments, each tube was observed for a number of periods (always of the same length) for each piece of material, until it was evident that the rate of respiration had become practically constant. Several of the tubes were then used as controls, while to the others was added the sea water containing the

anesthetic. In every case tubes which contained only sea water showed that the apparatus was not responsible for any of the changes observed in the experiments in which tissue was used.

The solutions used in any one experiment were always of the same PH value, so that the results were comparable. Many of the anesthetics, especially when in small concentrations, do not appreciably affect the PH value of the sea water. A large number of carboys of sea water, obtained from Woods Hole at the same time, were filtered to remove organic matter in varying degrees, the unfiltered material settling to the bottom. When an anesthetic decreased the PH value only slightly, it was possible by a suitable selection of the sea water from the carboys to obtain a bottle of sea water of a PH value equal to that of the sea water containing the anesthetic.

Whenever large amounts of an anesthetic were used in the sea water, it was considered advisable to add concentrated sea water to bring the electrical conductivity of the solution up to that of sea water. This was done with each of the following solutions: sea water containing 16.1 and 24.2 per cent alcohol respectively; sea water containing 17.4 per cent acetone; and sea water containing 3.2 per cent formaldehyde. Many of the experiments were repeated at Woods Hole in the summer of 1917. In these experiments the solutions containing alcohol (all above 1 per cent) were made of the same electrical conductivity as sea water. The results were practically the same as when no concentrated sea water was added.

In the case of the higher concentration of formaldehyde, the free acid was first neutralized with sodium carbonate. This is allowable for the purposes of the present investigation, as its only effect would be to make the amount of CO_2 produced appear somewhat less than was actually the case. It should be understood that the percentages of liquid anesthetics in the following descriptions are percentages by volume. In an experiment in which there is considerable dilution, as when absolute alcohol is added to sea water to make a 10 per cent alcoholic solution, it might be conceivable that the dilution might be the cause of the increased rate of respiration. For that reason experiments were made with

pieces of material in normal sea water until constant values for the respiration were obtained; then sea water diluted with 10 per cent of tap water was used, and it was found that the dilution produced no appreciable effect.

In each experiment 6 cc. of solution was used. At the end of each period, after noting the new PH value, 6 cc. of fresh solution was poured into the tube, which was then treated as before. It was at first thought that in pouring out the solution from the tube containing the tissue sufficient might be held back by the tissue to affect the observed PH value. Actual determinations proved that this was not the case.

In view of the fact that there may be an increase of respiration as the result of injury (23), preliminary experiments were made to ascertain whether the cutting of the *Laminaria* had any appreciable effect upon the respiration. It was found that the change in the respiration due to cutting was negligible (the cutting was always reduced to a minimum).

In some of the experiments there was noted a very slight decrease in the production of CO_2 as time went on, although not enough to be of significance for the present investigation. This phenomenon has been observed by Miss MATTHAEI (17) in determinations of CO_2 in connection with experiments on photosynthesis. Such a decrease in respiration has been attributed by her to the gradual decrease of substances available for oxidation.

The results of the experiments are given both in tables and figures. In each experiment 6–12 (or more) closely agreeing determinations were obtained and the results of a typical case were taken. The plant was always placed for a definite time in sea water or sea water containing the reagent, and at the end of this time the PH value was determined. This interval is in every case shown in the tables as well as by the points on the curves. At the end of each interval the solution was renewed.

The alteration of the PH value is an index of the amount of CO_2 produced, since the greater the amount of CO_2 the greater the decrease in PH. Since this relation is approximately linear in this range of PH values, it is not necessary to translate the decrease in PH value into cc. of CO_2 produced, as the form of the respiration

curve would be practically the same whether we use as ordinates PH values or cc. of CO_2 produced. The curves are plotted in such a manner that the abscissae represent time (in minutes) and the ordinates represent the change in PH value corresponding to either the relative amount of respiration, the relative rate of respiration, or to both (as designated in the figures). The relative amount of respiration is obtained by dividing the total change in PH during exposure to the reagent by the total change in PH caused by the

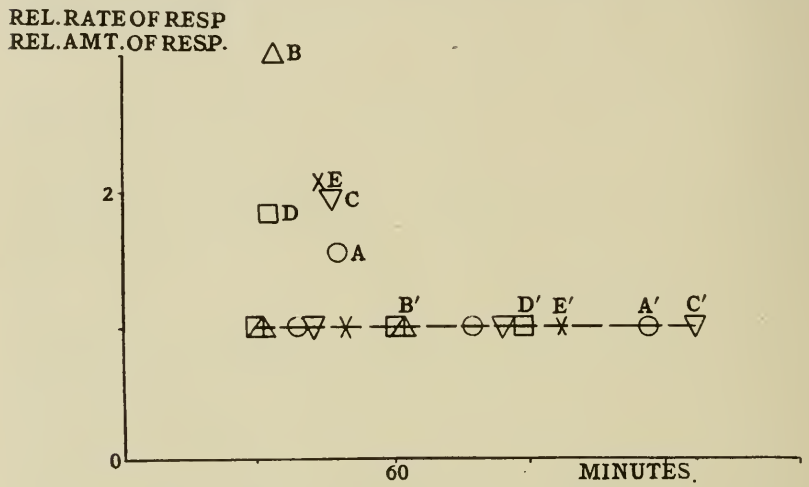

FIG. 1*a*.—Points showing relative rate and relative amount (identical in this case) of respiration of *Laminaria* produced by sea water containing *A*, 0.1 per cent chloral hydrate; *B*, 0.1 per cent novocain; *C*, 1 per cent ether; *D*, 0.1 per cent caffeine; *E*, ethyl-bromide; controls in sea water (broken lines); see tables I A to E; each control bears the same letter (with a prime) as the experimental curve.

same material under normal conditions during the same length of time. The relative rate is obtained by dividing the change in PH during one period by the change produced during a similar period by the same material under normal conditions. The broken lines in each case represent controls in sea water. The curve of each control bears the same symbol and letter as the experimental curve for sea water plus anesthetic, except that in the control the letters are primed.

Inspection of the results for 0.1 per cent chloral hydrate (fig. 1*a* [A]; table I A), 0.1 per cent novocain (fig. 1*a* [B]; table I B),

TABLE I A*

PERIOD OF 47.75 MIN. IN SEA WATER AND EQUAL PERIOD IN SEA WATER CONTAINING 0.1 PER CENT CHLORAL HYDRATE

Solution	Change in PH	Relative rate of respiration
Sea water	7.90−7.55=0.35	
Sea water containing 0.1 per cent chloral hydrate	7.90−7.35=0.55	0.55÷0.35=1.57

* In the control, with 3 periods (38.75 min. each) in sea water, the change in PH in each period was 7.90−7.55=0.35.

TABLE I B*

PERIOD OF 33.25 MIN. IN SEA WATER FOLLOWED BY EQUAL PERIOD IN SEA WATER CONTAINING 0.1 PER CENT NOVOCAIN

Solution	Change in PH	Relative rate of respiration
Sea water	7.65−7.40=0.25	
Sea water containing 0.1 per cent novocain	7.65−6.90=0.75	0.75÷0.25=3

* In the control, with 2 periods (30.75 min. each) in sea water, the change in PH in each period was 7.65−7.40=0.25.

TABLE I C*

PERIOD OF 45.25 MIN. IN SEA WATER AND EQUAL PERIOD IN SEA WATER CONTAINING 1 PER CENT (BY VOLUME) OF ETHER

Solution	Change in PH	Relative rate of respiration
Sea water	7.90−7.57=0.33	
Sea water containing 1 per cent ether	7.90−7.25=0.65	0.65÷0.33=1.97

* In the control, with 3 periods (42 min. each) in sea water, the change in PH in each period was 7.90−7.60=0.30.

TABLE I D*

TWO PERIODS (32.5 MIN. EACH) IN SEA WATER AND SAME LENGTH OF TIME IN SEA WATER CONTAINING 0.1 PER CENT CAFFEINE

Solution	Change in PH	Relative rate of respiration
Sea water	7.65−7.43=0.22	
" "	7.65−7.43=0.22	
Sea water containing 0.1 per cent caffeine	7.65−7.25=0.40	0.40÷0.22=1.8

* In the control, with 3 periods (29.5 min. each) in sea water, the change in PH in each period was 7.65−7.50=0.15.

TABLE I E*

PERIOD (43.25 MIN.) IN SEA WATER FOLLOWED BY EQUAL PERIOD IN SEA WATER APPROXIMATELY SATURATED WITH ETHYL BROMIDE

Solution	Change in PH	Relative rate of respiration
Sea water	7.90−7.35=0.55	
Sea water saturated with ethyl bromide	7.90−6.75=1.15	1.15÷0.55=2.1

* In the control, with 2 periods (49.25 min. each) in sea water, the change in PH in each period was 7.90−7.30=0.60.

TABLE I F

THREE PERIODS (22 MIN. EACH) IN SEA WATER AND 7 EQUAL PERIODS IN SEA WATER APPROXIMATELY SATURATED WITH ETHYL BROMIDE

Solution	Change in PH	Relative rate of respiration
Sea water	8.1 −7.8 =0.30	
" "	8.1 −7.8 =0.30	
Sea water containing ethyl bromide	7.32−6.16*=1.16+	1.16÷0.30=3.8
" " " " "	7.32−6.16*=1.16+	1.16÷0.30=3.8
" " " " "	7.32−6.69 =0.63	0.63÷0.30=2.1
" " " " "	7.32−7.07 =0.25	0.25÷0.30=0.8
" " " " "	7.32−7.21 =0.11	0.11÷0.30=0.4
" " " " "	7.32−7.32 =0.0	0.0 ÷0.30=0.0

* Approximate (at this point the indicator is not very sensitive to slight changes in acidity).

TABLE I F CONTROL

SEVEN PERIODS (27.5 MIN.) IN SEA WATER

Period	Change in PH	Relative rate of respiration
1	8.1−7.6 =0.5	0.5 ÷0.5=1.00
2	8.1−7.6 =0.5	0.5 ÷0.5=1.00
3	8.1−7.6 =0.5	0.5 ÷0.5=1.00
4	8.1−7.6 =0.5	0.5 ÷0.5=1.00
5	8.1−7.65=0.45	0.45÷0.5=0.90
6	8.1−7.65=0.45	0.45÷0.5=0.90
7	8.1−7.65=0.45	0.45÷0.5=0.90

1 per cent ether (fig. 1*a* [C]; table I C), 0.1 per cent caffeine (fig. 1*a* [D]; table I D), and for sea water approximately saturated with ethyl bromide (fig. 1*a* [E]; table I E; fig. 1*b* [A and B]; table I F) shows that these anesthetics increase both the relative amount and relative rate of respiration.

In fig. 1*b* (A) it will be seen that in sea water containing ethyl bromide the relative rate of respiration is greatly increased during the first 2 periods and then drops to the normal rate in about 90 min. After 132 min. no excretion of CO_2 could be detected. When we plot the curve B (fig. 1*b*), in which the ordinates represent the relative amount of respiration, we find that the curve is far above the base line even when the respiration cannot be detected.

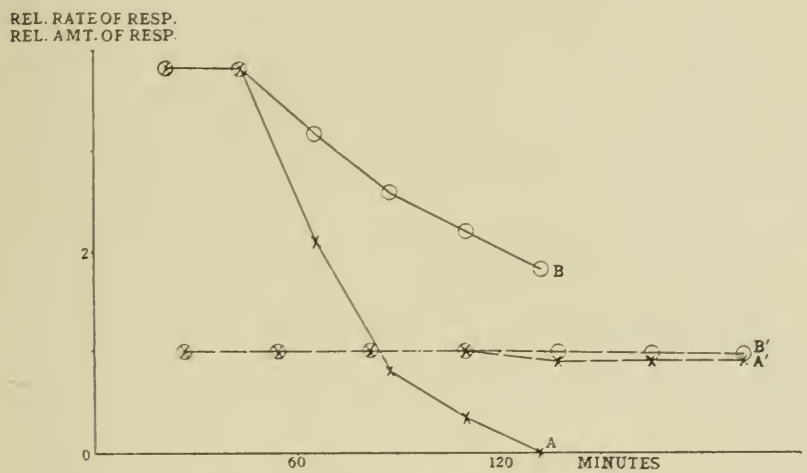

FIG. 1*b*.—Curves showing effect of sea water approximately saturated with ethyl bromide upon *A*, relative rate of respiration; B, relative amount of respiration of *Laminaria* (unbroken lines); controls in sea water (broken lines); see table I F; each control bears the same letter (with a prime) as the experimental curve.

The fact that ethyl bromide appears to have a marked accelerating effect upon the respiration confirms the results of APPLEMAN (1), who observed that when potato tubers are exposed to ethyl bromide vapor the respiration is greatly increased. Fig. 1*b* makes it evident that there is no initial decrease in the respiration.

The ethyl bromide referred to in table I F was acid, and since it was not neutralized by adding sodium carbonate it caused the PH of the sea water plus anesthetic to be lower than that of the sea water alone. In experiments with all other substances both sea water and sea water plus anesthetic were of the same PH value at the beginning of the experiment.

The respiration of *Laminaria* was followed for several hours, when the tissue was placed in solutions of sea water containing 3.2 per cent (fig. 2*a* [A], fig. 2*b* [A]; table II A) and 0.8 per cent

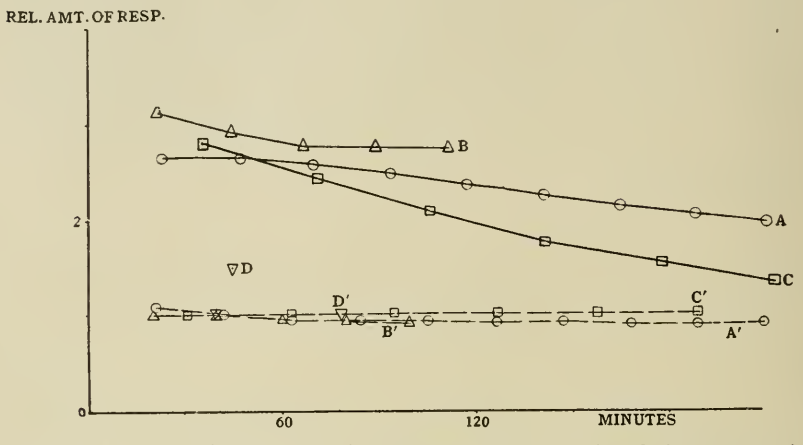

FIG. 2*a*.—Curves (unbroken lines) showing the effect upon the relative amount of respiration of *Laminaria* of sea water containing *A*, 3.2 per cent formaldehyde; *B*, 0.8 per cent formaldehyde; *C*, 0.3 per cent chloroform; *D*, 0.05 per cent chloroform; controls in sea water (broken lines); see tables II A to II D; each control bears the same letter (with a prime) as the experimental curve.

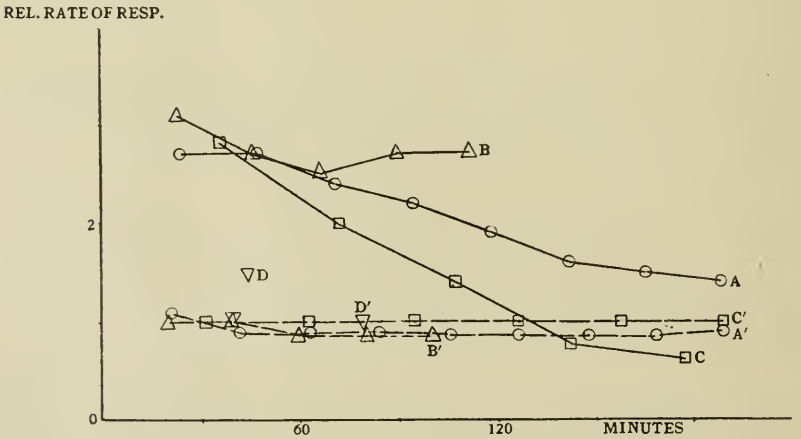

FIG. 2*b*.—Curves (unbroken lines) showing effect upon relative rate of respiration of *Laminaria* of sea water containing *A*, 3.2 per cent formaldehyde; *B*, 0.8 per cent formaldehyde; *C*, 0.3 per cent chloroform; *D*, 0.05 per cent chloroform; controls in sea water (broken lines); each control bears the same letter (with a prime) as the experimental curve.

TABLE II A

TWO PERIODS (23.5 MIN. EACH) IN SEA WATER AND 13 EQUAL PERIODS IN SEA WATER CONTAINING 3.2 PER CENT FORMALDEHYDE (8 PER CENT BY VOLUME OF 40 PER CENT FORMALDEHYDE)

Solution	Change in PH	Relative rate of respiration
Sea water	8.37−7.82=0.55	
" "	8.37−7.82=0.55	
Sea water containing 3.2 per cent formaldehyde	8.37−6.90=1.47	1.47÷0.55=2.7
Sea water containing 3.2 per cent formaldehyde	8.37−6.90=1.47	1.47÷0.55=2.7
Sea water containing 3.2 per cent formaldehyde	8.37−7.05=1.32	1.32÷0.55=2.4
Sea water containing 3.2 per cent formaldehyde	8.37−7.15=1.22	1.22÷0.55=2.2
Sea water containing 3.2 per cent formaldehyde	8.37−7.33=1.04	1.04÷0.55=1.9
Sea water containing 3.2 per cent formaldehyde	8.37−7.50=0.87	0.87÷0.55=1.6
Sea water containing 3.2 per cent formaldehyde	8.37−7.55=0.82	0.82÷0.55=1.5
Sea water containing 3.2 per cent formaldehyde	8.37−7.58=0.79	0.79÷0.55=1.4
Sea water containing 3.2 per cent formaldehyde	8.37−7.60=0.77	0.77÷0.55=1.4
Sea water containing 3.2 per cent formaldehyde	8.37−7.65=0.72	0.72÷0.55=1.3
Sea water containing 3.2 per cent formaldehyde	8.37−7.70=0.67	0.67÷0.55=1.2
Sea water containing 3.2 per cent formaldehyde	8.37−7.80=0.57	0.57÷0.55=1.0
Sea water containing 3.2 per cent formaldehyde	8.37−7.85=0.52	0.52÷0.55=0.95

TABLE II A CONTROL

FIFTEEN PERIODS (21 MIN.) IN SEA WATER

Period	Change in PH	Relative rate of respiration
1	8.37−7.80=0.57	0.57÷0.52=1.10
2	8.37−7.90=0.47	0.47÷0.52=0.90
3	8.37−7.90=0.47	0.47÷0.52=0.90
4	8.37−7.90=0.47	0.47÷0.52=0.90
5	8.37−7.92=0.45	0.45÷0.52=0.87
6	8.37−7.92=0.45	0.45÷0.52=0.87
7	8.37−7.92=0.45	0.45÷0.52=0.87
8	8.37−7.92=0.45	0.45÷0.52=0.87
9	8.37−7.90=0.47	0.47÷0.52=0.90
10	8.37−7.90=0.47	0.47÷0.52=0.90
11	8.37−7.90=0.47	0.47÷0.52=0.90
12	8.37−7.90=0.47	0.47÷0.52=0.90
13	8.37−7.90=0.47	0.47÷0.52=0.90
14	8.37−7.90=0.47	0.47÷0.52=0.90
15	8.37−7.90=0.47	0.47÷0.52=0.90

TABLE II B

TWO PERIODS (22.25 MIN. EACH) IN SEA WATER FOLLOWED BY 5 EQUAL PERIODS IN SEA WATER CONTAINING 0.8 PER CENT FORMALDEHYDE (2 PER CENT BY VOLUME OF 40 PER CENT FORMALDEHYDE)

Solution	Change in PH	Relative rate of respiration
Sea water	7.90−7.55=0.35	
" "	7.90−7.55=0.35	
Sea water containing 0.8 per cent formaldehyde	7.90−6.81=1.09	1.09÷0.35=3.1
Sea water containing 0.8 per cent formaldehyde	7.90−6.96=0.94	0.94÷0.35=2.7
Sea water containing 0.8 per cent formaldehyde	7.90−7.03=0.87	0.87÷0.35=2.5
Sea water containing 0.8 per cent formaldehyde	7.90−6.95=0.95	0.95÷0.35=2.7
Sea water containing 0.8 per cent formaldehyde	7.90−6.95=0.95	0.95÷0.35=2.7

TABLE II B CONTROL

FIVE PERIODS (20 MIN.) IN SEA WATER

Period	Change in PH	Relative rate of respiration
1	7.90−7.50=0.40	0.40÷0.40=1.00
2	7.90−7.50=0.40	0.40÷0.40=1.00
3	7.90−7.55=0.35	0.35÷0.40=0.87
4	7.90−7.55=0.35	0.35÷0.40=0.87
5	7.90−7.55=0.35	0.35÷0.40=0.87

TABLE II C*

TWO PERIODS (35.5 MIN. EACH) IN SEA WATER AND DURING 6 EQUAL PERIODS IN SEA WATER CONTAINING 0.3 PER CENT (BY VOLUME) OF CHLOROFORM

Solution	Change in PH	Relative rate of respiration
Sea water	8.03−7.43 =0.60	
" "	8.03−7.40 =0.63	
Sea water containing 0.3 per cent chloroform	8.03−6.30†=1.73	1.73÷0.615=2.8
Sea water containing 0.3 per cent chloroform	8.03−6.78 =1.25	1.25÷0.615=2.0
Sea water containing 0.3 per cent chloroform	8.03−7.15 =0.88	0.88÷0.615=1.4
Sea water containing 0.3 per cent chloroform	8.03−7.55 =0.48	0.48÷0.615=0.78
Sea water containing 0.3 per cent chloroform	8.03−7.65 =0.38	0.38÷0.615=0.62
Sea water containing 0.3 per cent chloroform	8.03−7.85 =0.18	0.18÷0.615=0.29

* In the control, with 8 periods (31.5 min. each) in sea water, the change in PH in each period was 8.03−7.80=0.23.

† Approximate (at this point the indicator is not very sensitive to changes in PH).

TABLE II D*

ONE PERIOD (44.5 MIN.) IN SEA WATER FOLLOWED BY EQUAL PERIOD IN SEA WATER CONTAINING 0.5 PER CENT (BY VOLUME) OF CHLOROFORM

Solution	Change in PH	Relative rate of respiration
Sea water	8.10—7.35=0.75	
Sea water containing 0.05 per cent chloroform	8.10—6.97=1.13	1.13÷0.75=1.5

* In the control, with 2 periods (39.25 min. each) in sea water, the change in PH in each period was 8.10—7.35=0.75.

(fig. 2*a* [B], fig. 2*b* [B]; table II B) of formaldehyde respectively. In both figs. 2*a* and 2*b* (A) and (B), there is a marked increase in the respiration during the first period. The curves for the weaker concentration tend to become approximately horizontal in the later periods. The curves for the stronger concentration of formaldehyde present a somewhat different case. Here the respiration reaches its maximum during the first period and maintains this rate during the second period. Following this, the respiratory rate steadily becomes smaller.

The curves for 0.3 per cent (fig. 2*a* [C] and fig. 2*b* [C]; table II C) and 0.5 per cent (fig. 2*a* [D] and fig. 2*b* [D]; table II D) chloroform respectively, each show that respiration is increased during the first period. The curves for the 0.3 per cent chloroform indicate that the rate steadily becomes smaller, until at the end of about 2.25 hours the respiratory rate falls below what it was normally (when in sea water).

The observation has often been made (11) that in human beings and in mammals during prolonged anesthesia there are typical products of incomplete oxidation such as fatty acids, lactic acid, and above all acetone (in not inconsiderable quantities) eliminated, as the case may be, into the urine or into the respired air. It seemed of interest in this connection to study the effect of acetone upon the respiration of *Laminaria*. It will be seen from the curves that when sea water contains 0.1 per cent (fig. 3 [A]; table III A) or 0.51 per cent (fig. 3 [B]; table III B) of acetone respectively, the respiration is practically unaffected. When, however, the sea water contains 17.4 per cent of acetone (fig. 3 [C] and [D]; table III C), a peculiar condition results. During the first period the

respiratory rate (curve D) is greatly increased and reaches its maximum during the second period. This is followed by a rapid decrease in the rate during the third and fourth periods, although the rate is still above the normal. After the fourth period a more

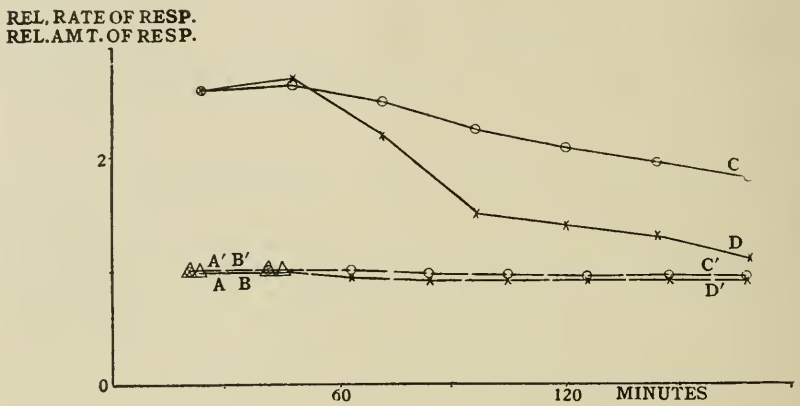

FIG. 3.—Curves (unbroken lines) showing effect of sea water containing *A*, 0.1 per cent acetone; *B*, 0.51 per cent acetone upon relative rate and relative amount of respiration of *Laminaria* (identical for these substances); *C*, effect of sea water containing 17.4 per cent acetone (unbroken line) upon relative amount of respiration; *D*, effect of sea water containing 17.4 per cent acetone (unbroken line) upon relative rate of respiration; controls in sea water (broken lines); see tables III A to III C; each control bears the same letter (with a prime) as the experimental curve.

gradual decline begins, so that even at the end of the experiment covering 2 hours and 48 min. the respiratory rate is still slightly above the normal.

TABLE III A AND B

TWO PERIODS (23 MIN. EACH) IN SEA WATER FOLLOWED BY 2 EQUAL PERIODS IN SEA WATER CONTAINING 0.1 PER CENT ACETONE, FOLLOWED BY 2 SIMILAR PERIODS IN SEA WATER CONTAINING 0.51 PER CENT ACETONE

Solution	Change in PH	Relative rate of respiration
Sea water	8.37−7.64=0.73	
" "	8.37−7.64=0.73	
Sea water containing 0.1 per cent acetone	8.37−7.65=0.72	0.72÷0.73=0.99
Sea water containing 0.1 per cent acetone	8.37−7.65=0.72	0.72÷0.73=0.99
Sea water containing 0.51 per cent acetone	8.37−7.65=0.72	0.72÷0.73=0.99
Sea water containing 0.51 per cent acetone	8.37−7.63=0.74	0.74÷0.73=1.01

TABLE III A AND B CONTROL

SIX PERIODS (20.25 MIN. EACH) IN SEA WATER

Period	Change in PH	Relative rate of respiration
1	8.37−7.60=0.77	0.77÷0.77=1.00
2	8.37−7.60=0.77	0.77÷0.77=1.00
3	8.37−7.61=0.76	0.76÷0.77=0.99
4	8.37−7.60=0.77	0.77÷0.77=1.00
5	8.37−7.60=0.77	0.77÷0.77=1.00
6	8.37−7.60=0.77	0.77÷0.77=1.00

TABLE III C

TWO PERIODS (24 MIN. EACH) IN SEA WATER AND 7 EQUAL PERIODS IN SEA WATER CONTAINING 17.4 PER CENT (BY VOLUME) OF ACETONE

Solution	Change in PH	Relative rate of respiration
Sea water	8.37−7.76=0.61	.....
" "	8.37−7.78=0.59	.....
Sea water containing 17.4 per cent acetone	8.37−6.80=1.57	1.57÷0.60=2.6
Sea water containing 17.4 per cent acetone	8.37−6.75=1.62	1.62÷0.60=2.7
Sea water containing 17.4 per cent acetone	8.37−7.06=1.31	1.31÷0.60=2.2
Sea water containing 17.4 per cent acetone	8.37−7.50=0.87	0.87÷0.60=1.5
Sea water containing 17.4 per cent acetone	8.37−7.54=0.83	0.83÷0.60=1.4
Sea water containing 17.4 per cent acetone	8.37−7.62=0.75	0.75÷0.60=1.3
Sea water containing 17.4 per cent acetone	8.37−7.74=0.63	0.63÷0.60=1.1

TABLE III C CONTROL

NINE PERIODS (21 MIN. EACH) IN SEA WATER

Period	Change in PH	Relative rate of respiration
1	8.37−7.67=0.70	0.70÷0.70=1.00
2	8.37−7.67=0.70	0.70÷0.70=1.00
3	8.37−7.70=0.67	0.67÷0.70=0.95
4	8.37−7.73=0.64	0.64÷0.70=0.91
5	8.37−7.73=0.64	0.64÷0.70=0.91
6	8.37−7.73=0.64	0.64÷0.70=0.91
7	8.37−7.72=0.65	0.65÷0.70=0.93
8	8.37−7.73=0.64	0.64÷0.70=0.91
9	8.37−7.72=0.65	0.65÷0.70=0.93

In view of the fact that alcohol is considered to be formed during respiration, it was deemed important to study the effect of varying concentrations of alcohol upon the rate of respiration. When sea water contains 1 per cent of Squibb's absolute alcohol (figs. 4*a* [F] and 4*b* [F]; table IV F), the respiratory rate remains normal for 3 periods, after which there is a gradual decline to below

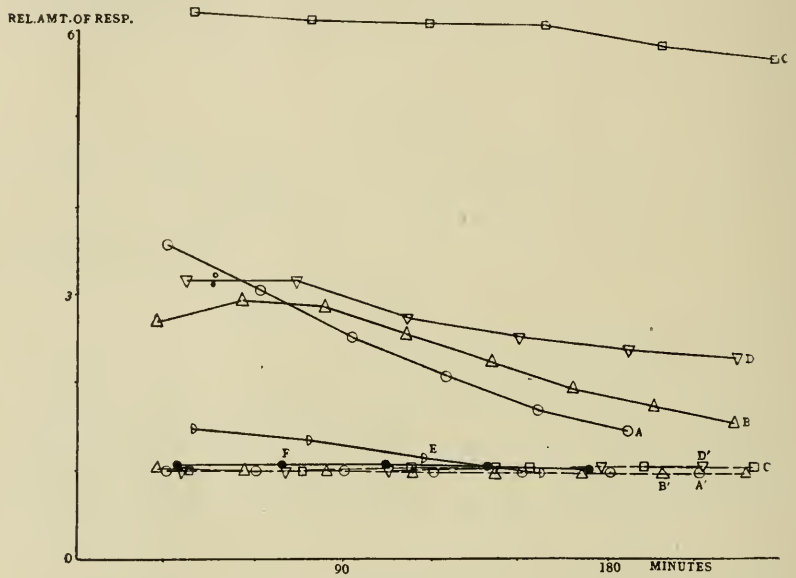

FIG. 4*a*.—Curves (unbroken lines) showing effect upon relative amount of respiration of *Laminaria* of sea water containing *A*, 24.2 per cent ethyl alcohol; *B*, 16.1 per cent ethyl alcohol; *C*, 10 per cent ethyl alcohol; *D*, 5 per cent ethyl alcohol; *E*, 2 per cent ethyl alcohol; *F*, 1 per cent ethyl alcohol; controls in sea water (broken lines); each control bears same letter (with prime) as the experimental curve, except that *D'* serves as control for curves *D*, *E*, and *F*; see tables IV A to IV F.

the normal rate, whereas the relative amount of respiration remains nearly constant. The curves for sea water containing 2 per cent alcohol (figs. 4*a* [E] and 4*b* [E]; table IV E) show a slight increase in the relative rate during the first period, followed by a smaller increase for the second period, after which there is a decline below the normal. As would be expected, 5 per cent alcohol (figs. 4*a* [D] and 4*b* [D]; table IV D) gives a much greater increase in the rela-

tive respiratory rate and amount than does 2 per cent alcohol (curve E). Unlike the 2 per cent alcohol, the maximum increase is maintained during the second period. The rate becomes less during the third period, after which it becomes quite constant. The curves for 10 per cent alcohol (figs. 4*a* [C] and 4*b* [C]; table IV C) reach their maximum during the first period. Approximately the

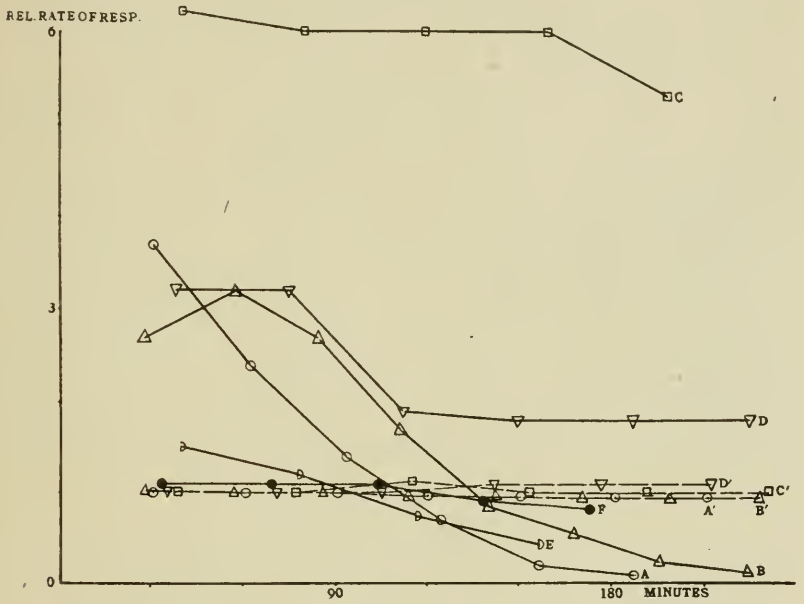

FIG. 4*b*.—Curves (unbroken lines) showing effect upon relative rate of respiration of *Laminaria* of sea water containing *A*, 24.2 per cent ethyl alcohol; *B*, 16.1 per cent ethyl alcohol; *C*, 10 per cent ethyl alcohol; *D*, 5 per cent ethyl alcohol; *E*, 2 per cent ethyl alcohol; *F*, 1 per cent ethyl alcohol; controls in sea water (broken lines); each control bears same letter (with prime) as the experimental curve, except that *D'* serves as control for curves *D*, *E*, and *F;* see tables IV A to IV F.

same rate of respiration is maintained for 3 periods, after which the increased rate rapidly becomes smaller. The relative amount of respiration remains approximately constant for 4 periods and then falls off very gradually. With 16.1 per cent alcohol (figs. 4*a* [B] and 4*b* [B]; table IV B) the maximum rate is not reached until the second period, after which the decline is more rapid than that for any of the lower concentrations. It will be seen that at the end of

224 min. the relative amount is about 1.5, while the relative rate falls below the normal after about 130 min. The maximum increased rate, when 24.2 per cent alcohol is used (figs. 4*a* [A] and 4*b* [A]; table IV A), is reached during the first period and then becomes smaller very much more rapidly than at any of the lower

TABLE IV A

TWO PERIODS (31.25 MIN. EACH) IN SEA WATER AND 6 EQUAL PERIODS IN SEA WATER CONTAINING 24.2 PER CENT (BY VOLUME) OF ETHYL ALCOHOL

Solution	Change in PH	Relative rate of respiration
Sea water	7.90−7.43 =0.47	.....
" "	7.90−7.43 =0.47	.....
Sea water containing 24.2 per cent ethyl alcohol	7.90−6.16*=1.74	1.74÷0.47=3.7
Sea water containing 24.2 per cent ethyl alcohol	7.90−6.75 =1.15	1.15÷0.47=2.4
Sea water containing 24.2 per cent ethyl alcohol	7.90−7.25 =0.65	0.65÷0.47=1.4
Sea water containing 24.2 per cent ethyl alcohol	7.90−7.55 =0.35	0.35÷0.47=0.7
Sea water containing 24.2 per cent ethyl alcohol	7.90−7.80 =0.10	0.10÷0.47=0.2
Sea water containing 24.2 per cent ethyl alcohol	7.90−7.84 =0.06	0.06÷0.47=0.1

* Approximate (at this point the indicator is not very sensitive to changes in PH).

TABLE IV A CONTROL

EIGHT PERIODS (30.25 MIN. EACH) IN SEA WATER

Period	Change in PH	Relative rate of respiration
1	7.90−7.53=0.37	0.37÷0.37=1.0
2	7.90−7.53=0.37	0.37÷0.37=1.0
3	7.90−7.53=0.37	0.37÷0.37=1.0
4	7.90−7.54=0.36	0.36÷0.37=0.97
5	7.90−7.54=0.36	0.36÷0.37=0.97
6	7.90−7.55=0.35	0.35÷0.37=0.95
7	7.90−7.55=0.35	0.35÷0.37=0.95
8	7.90−7.58=0.32	0.32÷0.37=0.87

concentrations, falling below the normal rate at about 110 min. The curve for relative amount of respiration at the end of 3 hours is about 1.4, even though respiration has nearly ceased.

These curves, showing the effect of varying concentrations of alcohol upon the rate of respiration, indicate that for concentrations above 1 per cent there is a marked increase in the respiratory

activity. Furthermore, there is a maximum increase which is usually reached in the first, but sometimes not until the second period. The relative amount and relative rate of respiration increase with increasing concentrations of ethyl alcohol up to 10

TABLE IV B

TWO PERIODS (28 MIN. EACH) IN SEA WATER FOLLOWED BY 8 EQUAL PERIODS IN SEA WATER CONTAINING 16.1 PER CENT ETHYL ALCOHOL

Solution	Change in PH	Relative rate of respiration
Sea water	7.95−7.50=0.45	
" "	7.95−7.52=0.43	
Sea water containing 16.1 per cent ethyl alcohol	7.95−6.76=1.19	1.19÷0.44=2.7
Sea water containing 16.1 per cent ethyl alcohol	7.95−6.55=1.40	1.4 ÷0.44=3.2
Sea water containing 16.1 per cent ethyl alcohol	7.95−6.75=1.20	1.2 ÷0.44=2.7
Sea water containing 16.1 per cent ethyl alcohol	7.95−7.20=0.75	0.75÷0.44=1.7
Sea water containing 16.1 per cent ethyl alcohol	7.95−7.57=0.38	0.38÷0.44=0.86
Sea water containing 16.1 per cent ethyl alcohol	7.95−7.70=0.25	0.25÷0.44=0.57
Sea water containing 16.1 per cent ethyl alcohol	7.95−7.85=0.10	0.10÷0.44=0.23
Sea water containing 16.1 per cent ethyl alcohol	7.95−7.90=0.05	0.05÷0.44=0.11

TABLE IV B CONTROL

NINE PERIODS (28.5 MIN. EACH) IN SEA WATER

Period	Change in PH	Relative rate of respiration
1	7.95−7.55=0.40	0.40÷0.395=1.01
2	7.95−7.56=0.39	0.39÷0.395=0.99
3	7.95−7.56=0.39	0.39÷0.395=0.99
4	7.95−7.57=0.38	0.38÷0.395=0.96
5	7.95−7.58=0.37	0.37÷0.395=0.94
6	7.95−7.58=0.37	0.37÷0.395=0.94
7	7.95−7.58=0.37	0.37÷0.395=0.94
8	7.95−7.58=0.37	0.37÷0.395=0.94
9	7.95−7.58=0.37	0.37÷0.395=0.94

per cent. At concentrations from 2 to 10 per cent the relative rate of respiration remained far above the normal during the entire experiment. When larger concentrations such as 16.1 or 24 per cent are used, however, the decline, once the maximum rate has been reached, becomes more rapid with increasing concentrations

of alcohol. The curves of the relative rate or respiration for such higher concentrations of alcohol fall quite rapidly below the normal, whereas the curves for the relative amount of respiration for the same experiment may remain far above unity.

TABLE IV C

TWO PERIODS (39.75 MIN. EACH) IN SEA WATER FOLLOWED BY 6 EQUAL PERIODS IN SEA WATER CONTAINING 10 PER CENT ETHYL ALCOHOL

Solution	Change in PH	Relative rate of respiration
Sea water	7.70−7.55=0.15	.....
" "	7.70−7.55=0.15	.....
Sea water containing 10 per cent ethyl alcohol	7.70−6.77=0.93	0.93÷0.15=6.2
Sea water containing 10 per cent ethyl alcohol	7.70−6.80=0.90	0.90÷0.15=6.0
Sea water containing 10 per cent ethyl alcohol	7.70−6.80=0.90	0.90÷0.15=6.0
Sea water containing 10 per cent ethyl alcohol	7.70−6.80=0.90	0.90÷0.15=6.0
Sea water containing 10 per cent ethyl alcohol	7.70−6.90=0.80	0.80÷0.15=5.3
Sea water containing 10 per cent ethyl alcohol	7.70−7.05=0.65	0.65÷0.15=4.3

TABLE IV C CONTROL

EIGHT PERIODS (38.5 MIN. EACH) IN SEA WATER

Period	Change in PH	Relative rate of respiration
1	7.70−7.55=0.15	0.15÷0.15=1.00
2	7.70−7.55=0.15	0.15÷0.15=1.00
3	7.70−7.53=0.17	0.17÷0.15=1.13
4	7.70−7.55=0.15	0.15÷0.15=1.00
5	7.70−7.55=0.15	0.15÷0.15=1.00
6	7.70−7.55=0.15	0.15÷0.15=1.00
7	7.70−7.55=0.15	0.15÷0.15=1.00
8	7.70−7.55=0.15	0.15÷0.15=1.00

It is important to know whether or not the decrease in PH value is due to the excretion of CO_2 or to other acids, such as organic acids which are products of incomplete oxidation. To determine this pure hydrogen was bubbled through the solution (which has been made more acid by *Laminaria*) until the excess of CO_2 was expelled. The solution was then allowed to come into equilibrium with the CO_2 of the air. It was found that in all the experiments

(with certain exceptions to be mentioned) the color of the indicator was reversible by this means. This showed conclusively that the increased acidity was actually due to CO_2. In the case of sea water containing 0.3 per cent chloroform, the color of the indicator

TABLE IV D

TWO PERIODS (37.5 MIN. EACH) IN SEA WATER FOLLOWED BY 6 EQUAL PERIODS IN SEA WATER CONTAINING 5 PER CENT ETHYL ALCOHOL

Solution	Change in PH	Relative rate of respiration
Sea water	7.65−7.43=0.22	
" "	7.65−7.43=0.22	
Sea water containing 5 per cent ethyl alcohol	7.65−6.95=0.70	0.70÷0.22=3.2
Sea water containing 5 per cent ethyl alcohol	7.65−6.95=0.70	0.70÷0.22=3.2
Sea water containing 5 per cent ethyl alcohol	7.65−7.22=0.43	0.43÷0.22=1.9
Sea water containing 5 per cent ethyl alcohol	7.65−7.25=0.40	0.40÷0.22=1.8
Sea water containing 5 per cent ethyl alcohol	7.65−7.25=0.40	0.40÷0.22=1.8
Sea water containing 5 per cent ethyl alcohol	7.65−7.25=0.40	0.40=0.22=1.8

TABLE IV E

TWO PERIODS (39.25 MIN. EACH) IN SEA WATER FOLLOWED BY 4 EQUAL PERIODS IN SEA WATER CONTAINING 2 PER CENT ETHYL ALCOHOL

Solution	Change in PH	Relative rate of respiration
Sea water	7.65−7.35=0.30	
" "	7.65−7.35=0.30	
Sea water containing 2 per cent ethyl alcohol	7.65−7.20=0.45	0.45÷0.30=1.5
Sea water containing 2 per cent ethyl alcohol	7.65−7.28=0.37	0.37÷0.30=1.2
Sea water containing 2 per cent ethyl alcohol	7.65−7.43=0.22	0.22÷0.30=0.73
Sea water containing 2 per cent ethyl alcohol	7.65−7.52=0.13	0.13÷0.30=0.43

was not reversible at the end of the first period, although after any of the succeeding periods the color was fully reversible. The color of the indicator in sea water containing 17.4 per cent acetone (made up to the conductivity of sea water) was not reversible at the end of the first period of exposure. At the end of the second period of

exposure the color was almost entirely reversible, and after the third period (and succeeding periods) the color was completely reversible. When sea water containing 24.2 per cent ethyl alcohol (made up to conductivity of sea water) was used, the color of the indicator after the first period of exposure was not reversible. The

TABLE IV F

TWO PERIODS (34.75 MIN. EACH) IN SEA WATER FOLLOWED BY 5 EQUAL PERIODS IN SEA WATER CONTAINING 1 PER CENT ETHYL ALCOHOL

Solution	Change in PH	Relative rate of respiration
Sea water	7.65−7.40=0.25	.....
" "	7.65−7.35=0.30	.....
Sea water containing 1 per cent ethyl alcohol	7.65−7.35=0.30	0.30÷0.275=1.1
Sea water containing 1 per cent ethyl alcohol	7.65−7.35=0.30	0.30÷0.275=1.1
Sea water containing 1 per cent ethyl alcohol	7.65−7.35=0.30	0.30÷0.275=1.1
Sea water containing 1 per cent ethyl alcohol	7.65−7.40=0.25	0.25÷0.275=0.91
Sea water containing 1 per cent ethyl alcohol	7.65−7.43=0.22	0.22÷0.275=0.80

TABLE IV D, E, F CONTROL

SIX PERIODS (35.5 MIN. EACH) IN SEA WATER

Period	Change in PH	Relative rate of respiration
1	7.65−7.45=0.20	0.20÷0.20=1.00
2	7.65−7.45=0.20	0.20÷0.20=1.00
3	7.65−7.45=0.20	0.20÷0.20=1.00
4	7.65−7.43=0.22	0.22÷0.20=1.10
5	7.65−7.43=0.22	0.22÷0.20=1.10
6	7.65−7.43=0.22	0.22÷0.20=1.10

color of the indicator at the end of the second period of exposure was almost completely reversible, but after each subsequent period the color was fully reversible.

The reason for these instances of irreversibility can easily be explained. Phenolsulphonephthalein in the acid end of its range is yellow. *Laminaria* has a yellowish brown pigment which may come out when the cells are injured by very strong concentrations of the anesthetic, while the green pigment does not come out. When the concentration of the anesthetic is so great as to rapidly injure the cells (as is the case with all of the exceptions just noted),

the yellowish pigment comes out so rapidly as to interfere with the indicator. As soon as the extraction of the pigment ceases (usually ending during the first 2 periods), the color of the indicator is not interfered with. When the concentrations of anesthetics are lower than in those instances just mentioned, the pigment comes out (if at all) so slowly as not to affect the indicator. This can be proven by matching the solution obtained after any period (without the addition of indicator) with the color of sea water (containing no indicator) in a tube of equal diameter. In such cases no pigment is detectable.

Further evidence that it is CO_2 that is being measured in the experiments rather than other acids is the fact that by the use of the gas chain it was found that *Laminaria*, after being 2 weeks in a small quantity of unchanged sea water, had given off no acid other than CO_2.

It might be supposed that the addition of so much alcohol as 24.2 per cent would dilute the buffer substances of the sea water so that a given amount of CO_2 added to the mixture would produce more change in PH value than would be the case in sea water. This was largely avoided by concentrating the sea water before adding the alcohol, so that the amount of buffer substance remained the same in the mixture as in the sea water alone. Tests made by adding measured amounts of CO_2 to sea water and to sea water plus 24.2 per cent alcohol (made up to the electrical conductivity of sea water) showed that there was not sufficient difference in this respect to be of importance in this investigation.

Such experiments enable us to follow the respiration of the same piece of tissue during shorter or longer periods of exposure to various concentrations of anesthetics. They further show that in no instance was there an initial decrease in the rate of respiration.

It will be observed that when the concentration of anesthetic is strong enough to produce any measurable result, the first effect is an increase of respiration, which gradually declines and may eventually fall below the normal. This decline is interpreted by the writer as a toxic effect.

These results are not in accord with the statements of TASHIRO and ADAMS (27), according to whom anesthetics do not produce

an increase of respiration except when their concentration is so low that they have only a stimulating action. They state that when the concentration is increased to the point where anesthesia occurs, the rate of respiration falls below the normal.

It is evident that this is not the case with *Laminaria*, for in no instance was the respiration observed to fall below the normal except after prolonged exposure to high concentrations which produced death. Further investigation will be necessary to determine the cause of these discrepancies.

It is evident that these experiments directly contradict the idea, advocated by VERWORN (29) and his pupils, that anesthesia is a kind of asphyxia and that anesthetics act by reducing respiration.

Summary

When *Laminaria* is exposed to anesthetics (in sufficiently high concentration to produce any result) the initial effect is an increase of respiration. This may be followed by a decrease if the anesthetic is sufficiently toxic. No decrease of respiration is observed when the concentration is too low to be toxic.

These results directly contradict the idea advocated by VERWORN and his pupils that anesthetics act by decreasing respiration.

LABORATORY OF PLANT PHYSIOLOGY
HARVARD UNIVERSITY

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BASIS OF SUCCULENCE IN PLANTS

D. T. MacDougal, H. M. Richards, and H. A. Spoehr

Succulents may be characterized as plants in which the parenchymatous elements show an exaggerated development with relation to the more rigid tissues, and, unlike pith or medullary tracts, the masses of thin-walled cells remain distended and turgid. The liquid contents of such cells may or may not contain much dissolved material. The disposition of the water-holding tracts varies from leaves to stems and roots, but in all cases the most important general effect is one of massiveness, and the surfaces of succulent plants may in such forms as the barrel cacti bear the smallest possible proportion to the mass, that of a globe.

The ecologist recognizes two general types of succulents, those of the arid regions, which are of a xerophytic character, exemplified by the cacti; and the halophytes or fleshy seashore plants, also at home in alkaline areas. The plants of the two types are quite unlike in their transpiratory relations. The desert succulents may lose water so slowly that an existence of several years may be maintained upon the water in the thin-walled tracts.[1] On the other hand, the halophytes or fleshy shore plants may flag and wilt as readily as any thin-leaved form, due to the rapid loss of water from the surfaces. The origination of these striking forms has been the subject of much speculation, but all attempts to connect succulency in a causal way with the presence of salts in the soil, or in the plant with the well known high acidity of many of these forms, or with any purposeful development of water storage capacity, have been inadequate.

Our concurrent observations and experiments may be briefly summarized as follows:

1. A *Castilleja* native to the region about the Coastal Laboratory, at Carmel, California, includes two habitat forms, genetically identical, one with thin leaves growing in the open forest formation,

[1] MacDougal, D. T., et al., End results of desiccation and respiration in succulent plants. Physiol. Researches 1: 289–325. 1915.

and another with fleshy leaves growing on the sandy foreshores under arid, but not saline, soil conditions. The succulent leaves owe their increased thickness to the enlargement of elongated cells vertical to the surface of the leaves.

2. The thin leaves show an acidity double that of the fleshy type, and have a relatively greater dry weight.

3. The fleshy leaves, fresh and in a dried condition, present swelling reactions similar to those of sections of the joints of platyopuntias, indicative of cells high in pentosans, or mucilages. The behavior of these organs is different in many important particulars from that of thin leaves, which swell more in acid than in alkaline solutions, the reverse taking place in succulent leaves, in parallelism to *Opuntia*.

4. Differences in the swelling reactions of dried leaves of both kinds are to be ascribed to the adsorption of the contained acids and salts of different amounts in the two cases on cell colloids, high in pentosans in one case and hence presenting characteristic coagulatory effects.

5. It has been established by researches not described in this paper that the reduction of the water content of the cell below a certain point results in the conversion of polysaccharides, which do not show a high imbibition capacity, to pentosans, which mixed with nitrogenous substances have an enormous hydration capacity.

6. Succulence, therefore, may originate as it is seen to occur in *Castilleja* as a direct result of aridity. Species of *Ericameria* and *Erigeron* with a distribution similar to *Castilleja* display thin and succulent leaves corresponding in the same manner to the environment.

7. High acidity may not be taken as a result of succulence. It is probably more nearly correct to assume that succulence may develop only in plants which have a carbohydrate metabolism characterized by large acid residues.

The bulk and durability of succulents have made them readily available for chemical studies, and these features are responsible in part for the fact that carbohydrate metabolism and respiration, photosynthesis, the formation and fate of acids, the oxygen-carbon-dioxide ratio, and other features have all received contributions

based upon researches carried out with the fleshy plants.[2] The amount of detailed and systematic information concerning these plants as a type is probably greater than that of any other ecological group, and it is evident that their metabolism presents some definite characteristic aspects. It is upon the basis of such knowledge that it becomes possible to formulate the generalizations set forth in this paper.

The only comparisons between succulents and non-succulents that have been possible have lacked directness because the reactions of different species could not be rated against each other with accuracy. The final and necessary conditions for a critical discussion of the matter, that of succulent and non-succulent individuals of the same species, finally came to the attention of the authors in the case of *Castilleja latifolia* at Carmel, California, in the summer of 1918. One form of this plant which grows on the edge of the bluff overlooking the beach, or within 25 ft. of it, has succulent leaves of considerable thickness which are usually pale green. The other, which grows farther back on the foreshore, has a thinner, mesophytic type of leaf which is darker green and more hirsute than the succulent type. This is probably the typical form of the manuals and is similar to the one which grows farther back in the pine woods. A notable exception as to the relative region of growth of these 2 forms was found in a luxuriant growth of the thin-leaved type at the base of the beach bluff on the edge of the sand. Examination showed that this was unquestionably due simply to the ample water supply from the seepage at the base of the cliff. It becomes evident, therefore, that the difference in the succulent and mesophytic habit is not a case of even partial halophytism, for if salt were present anywhere it would be at the cliff base. The contrasting habit is one rather of xerophytism versus a mesophytic growth.

The members of the genus are reported to be parasitic, and individuals with thin and others with succulent leaves were found

[2] SPOEHR, H. A., Photochemische Vorgänge bei der Diurnalen Entsäurung der Succulenten. Biochem. Zeitsch. **57**:95–111. 1913.

RICHARDS, H. M., Acidity and gas interchange in cacti. Publ. no. 209. Carnegie Inst. Wash. 1915.

HEMPEL, JENNY, Buffer process in the metabolism of succulent plants. Compt. Rend. Carlsberg. **13**:no. 1. 1917.

with minute roots attached to the older tapering roots of *Artemisia pycnocephala*, in a manner indicating that the dependent nutritive relation is not an important one. That the appearance of succulence in this plant has no connection with its parasitism is supported by the fact that a similar state was found in *Erigeron glaucus* and *Ericameria ericoides* which are found in the locality near *Castilleja*.

Measurements of the thickness of the leaves of the two types show that the thin leaf averages about 0.5 mm., while the succulent one ranges from 1 to 1.5 mm. Examination of sections shows that the structure of the leaves is mainly differentiated by the size of the cells. While nearly dorsiventral in position when young, structurally the leaves appear almost bifacial, with 2 or 3 rows of vertically elongated cells on each face. In the thin-leaved type these cells are about $35 \times 25\ \mu$, while similar cells of the succulent leaf are $110 \times 30\ \mu$. Thus the increased thickness of the leaf is due chiefly to the enlargement of the cells vertical to the flat surface of the leaf.

There is naturally a disparity in the relation of fresh to dry weight in the 2 forms. Averages of a number of determinations show the following figures: mesophytic type, 1 gm. fresh young leaves, 0.193 dry weight; succulent type, 1 gm. fresh young leaves, 0.113 dry weight. In general the succulent type yields only three-fifths dry substance per unit fresh substance compared with the other form.

The acidity relations are also different. The succulent leaves are much less acid than the thinner ones. In table I averages of 10 or more determinations indicate the difference in acid extracted, and also the amount of water absorbed after 24 hours' immersion. As might be expected, the acidity relation of the 2 forms approximates more closely when reckoned according to dry weight than on the basis of fresh weight. Even then, however, the young active leaves show a considerably greater acidity in the thin type, a notable departure from our preconceived conceptions of acidity in relation to succulence. It may be said, however, that direct comparisons of acidity of a plant of the same species in a mesophytic and succulent condition do not seem to have been made.

In connection with the swelling measurements described later, it became important to ascertain how much acid would leach out during immersion in water. Table II indicates the averages of a considerable number of series.

It is noticeable that the total amount of acid is somewhat greater than the figures previously given, due very possibly to the formation of acid during immersion in water, which might be caused

TABLE I

Material	Total acidity		Water absorbed, cc. per gm. fresh weight
	Per gm. fresh	Per gm. dry	
Thin young leaves	1.50 cc. N/20 KOH	7.78 cc. N/20 KOH	1.14
Succulent young leaves	0.62 N/20	5.37 N/20	0.57
Thin old leaves	1.00 N/20	5.20 N/20	0.85
Succulent old leaves	0.65 N/20	5.74 N/20	0.35

TABLE II

LEAVES IMMERSED IN WATER FOR 24 HOURS AT 17° C.

Material	Acid diffused out per gm. fresh weight	Acid retained in tissue per gm. fresh weight
Thin young leaves	1.30 cc. N/20 KOH	0.39 cc. N/20 KOH
Succulent young leaves	0.55 N/20	0.28 N/20
Thin old leaves	0.62 N/20	0.66 N/20
Succulent old leaves	0.30 N/20	0.41 N/20

by the exclusion of oxygen. It is also to be observed that the residual acid in the young leaves is closely the same in both the succulent and thin-leaved type. It appears that in the old leaves proportionately less of the acid leaches out and more is retained in the tissues. Other series of experiments were undertaken to determine the rate at which the acid diffuses out, the results of which are given in table III.

During the first hours of immersion the amount of acid which passes out is small and nearly equal in each case. As prolonged immersion in water kills the leaves, it seems probable that very little acid escapes as long as the cells are alive.[3] Table IV indicates

[3] LAUK, E., Die Bedeutung der Elektrolyten fuer Quellungsprocess. Biochem. Zeitsch. 37: 15–58. 1916.

the length of immersion which the 2 forms can withstand and recover to a seemingly normal condition.

After 12 hours' immersion in water all the thin-leaved shoots were killed; some of the succulent leaved shoots survived partly.

TABLE III

RATE OF DIFFUSION OF ACID IN TERMS OF CC. N/20 KOH PER GM. FRESH WEIGHT AT 17° C.

Material	3 hours	6 hours	9 hours	12 hours	15 hours	24 hours	Residual acid	Total
Thin young leaves.....	0.10	0.20	0.27	0.30	0.15	0.05	0.30	1.37
Succulent young leaves.....	0.10	0.12	0.18	0.15	0.05	Trace	0.23	0.80

TABLE IV

Material	1.5 hours	3 hours	6 hours	9 hours
Thin young leaves.....	All recovered	All recovered	Some killed	More than half killed
Succulent young leaves .	All recovered	All recovered	Some killed	About half killed

TABLE V

SWELLING OF LEAVES OF *Castilleja* AT 16° C.

Material	Water	Citric acid 0.01 N	Potassium hydrate 0.01 M	Potassium nitrate 0.01 M
	Percentage	Percentage	Percentage	Percentage
Thin.....	149	120	60	76
Succulent.....	143	95	125	90

The reactions of the succulent leaves are seen to present the general aspects of sections of joints of *Opuntia discata* grown at Carmel and tested at 16° C. at Carmel, July 1918. Swellings of dried slices are as follows:

Material	Water	Citric acid 0.01 N	Potassium hydrate 0.01 M	Potassium nitrate 0.01 M
	Percentage	Percentage	Percentage	Percentage
Fresh sections...	9	10	11.7	10.4
Dried slices.....	509	218	413	417

A set of sections were now prepared, and, after being swelled with reactions parallel to the preceding, were dried, and the expansion when immersed was calculated on the original thickness, as follows:

Material	Water	Citric acid 0.01 N	Potassium hydrate 0.01 M	Potassium nitrate 0.01 M
	Percentage	Percentage	Percentage	Percentage
Fresh sections ..	11.4	6.4	6.6	9.2
Dried sections ..	17.5	26.4	24.5	22.4

Determinations were also made of the escape of acid from dried leaves, which, as shown in table VI, is much more rapid than with the living material. It will be seen that the total of the acid extracted from the dried leaves is much less than that obtained from fresh leaves, which, as might be expected, indicates that some of the

TABLE VI

ESCAPE OF ACID FROM DRIED LEAVES IN TERMS OF CC. N/20 KOH PER GM. FRESH WEIGHT AT 17° C.

Material	2 hours	6 hours	12 hours	15 hours	24 hours	Residue	Total
Succulent young leaves..	0.30	0.10	0.5	Trace	Trace	0.12	0.57
Thin young leaves.....	0.54	0.20	0.8	Trace	Trace	0.40	1.22

acid salts are absorbed and held in the irreversible aggregation phenomena connected with the processes of drying. It may be mentioned that two series of both kinds of leaves, which by chance were dried much more slowly than the others, showed a difference in the rate at which they yielded up their acid; in both cases the amount which escaped in 2 hours was much less than in the case of the rapidly dried leaves.

When trios of leaves of the 2 types were placed under the auxograph to determine their unsatisfied hydration capacity, swellings as follows (table VII) were displayed at 16° C.

As will be seen by comparisons with data obtained from *Castilleja*, the dried mass behaves like the succulent leaves by showing but little expansion after immersion and drying.

Similar tests were applied to sections and to dried median slices of an unknown *Opuntia* which appeared to be less mucilaginous than *O. discata*. Dried slices came down to a thickness of

about 0.2 mm. and these gave swellings at 16° C. which are to be compared with the swellings of fresh material.

The second swelling produced an expansion scarcely more than half that of the first in all solutions, and being still further decreased in alkali, furnishing striking parallels with the action of succulent leaves of *Castilleja*.

TABLE VII

Process	Water	Citric acid 0.01 N	Potassium hydrate 0.01 M	Potassium nitrate 0.01 M
	Percentage	Percentage	Percentage	Percentage
After first drying	361	306	250	325
After second drying on basis of reduced thickness	42	56	100	75
Fresh sections through joints	9.7	7	.....	9.3

TABLE VIII

HYDRATION REACTIONS OF SUCCULENT AND THIN LEAVES OF *Castilleja*; JULY 28–31; AT 16° C.

Material	Water	Citric acid 0.01 N	Potassium hydrate 0.01 M	Potassium nitrate 0.01 M
	Percentage	Percentage	Percentage	Percentage
Succulent				
Fresh	143	95	125	90
Swelled and dried	20	16	20	60
Fresh dried	76	60	55	65
Thin leaves				
Fresh	140	120	60	76
Swelled and dried	132	12	67	153
Fresh dried	100	20	118	130

Two additional treatments of the leaves were given to test the effects of hydration on the swelling capacity of the contained colloids. In one case the trios of sections which had swelled were dried on filter paper for a day at 20° C., with only enough pressure to prevent warping or curling, then again hydrated in water or the identical solutions of the first swelling. The second case included a swelling of leaves which had been simply dried for a day at 20° C., in which process they came down to about half the original thickness. The measurements at 17–18° C., calculated on dried thickness, which was usually about one-half that of living material, are given in table VIII.

The comparisons which may be made upon the basis of such data are almost endless, and a citation of even the salient features of interest cannot be made briefly. The proportionate hydration of the succulent and thin leaves are reversed in acid and alkali. The succulent leaf, which proves to be one-half as acid as the thin leaf, swells most in the alkaline solution; while the thin leaves, with an acidity double that of the thick succulent ones, have an equivalent maximum in hundredth normal citric acid, and take up only half as much water in the alkaline solution, the disproportion between the two expansions being greater than that of the acid alkali ratio in the succulent.

The thin leaves are characterized by a uniformly high hydration capacity in water in the 3 cases, although reaching a maximum in the salt, a high swelling capacity in acid when fresh, which undergoes a great reduction after drying, while the swelling capacity increases in alkali in parallel treatments. The maximum swelling of the succulent leaves is in water, with great variation in the 3 conditions in which leaves were tested, and with but little variation in the reactions in the salt. The thin leaves, on the other hand, show the maximum and greatest diversity in the salts and more uniformity in water.

The variations in swelling in the acid solution presented such unusual features that an additional series was planned in which thin and succulent leaves in fresh condition were swelled, then such leaves fully hydrated in water and in various solutions were dried and swelled a second time for comparison with the reactions of leaves dried directly from the living condition. Table IX shows the swellings in 0.01 normal citric acid at 15° C.

The chief departure from the original series is in the matter of the swelling of the fresh succulent leaves, which in this case appear to have been in such a highly hydrated condition as only to be capable of slight expansion. This assumption is in accordance with the fact that after being immersed and then dried they assumed approximately the original thickness on a second swelling. The thin leaves of this series were consistent in their reactions with those previously examined, showing a relatively small expansion from a dried condition. The conditions making possible the greater variations are evidently those recognizable in the succulent type,

not the least important feature being the greatly enlarged parenchymatous cells. It is to be seen that immersion and drying, and also simple drying, reduce the swelling capacity of thin leaves in acid, but no such decrease occurs in the succulent leaf.

The principal changes which take place in swelling consist in the extraction of acids and acid salts, as indicated on the previous pages, and of the hexoses as yet undetermined. Any mucilages or pentosans present would of course diffuse at a rate so slow as to be of no consequence in the present experiments.

TABLE IX

Material	Thin		Succulent	
	Thickness	Swelling	Thickness	Swelling
	mm.	Percentage	mm.	Percentage
Fresh leaves	0.4	125	1.4	21
	0.41	184	1.4	25
Above leaves dried and rehydrated*	0.23	42	0.5–0.6	95
	0.25	20	0.63	91
Fresh dried leaves*	(0.38)	.....	(1.2)	.....
	0.2	25	0.5	120
	(0.38)	.....	(1.1)	.....
	0.2	62	0.38	92

* Expansion in terms of dried thickness.

The swelling of fresh leaves of both types in water reaches the limit in less than 2 hours, the rate of extraction of acid in the 2 types of leaves being equivalent, and the proportionate expansions not widely different. When such leaves are dried the thin leaves attain the limit in water inside of 2 hours, while the succulent leaves continue to expand for 6 hours with an escape of acid about half that from the thin leaf during the same time.

If attention be turned to the reactions in acids, it is seen that thin leaves swell more than succulents in such solutions when fresh, and that the swelling extends over a greater length of time, while the total swelling in a dried condition is accomplished in a few minutes. The succulent leaves, on the other hand, require a period of as much as 6 hours to reach full hydration from a dried condition.

While the effect of the residual acidity is discernible in some of these relations, it is evident that this factor is not the dominating

one. Two other features remain to be considered, that of the composition of the plasmatic colloids, and of the salts dissolved in the water of hydration. The colloids of living leaves are highly hydrated, and the salts, acids, etc., are also in a highly dilute condition, in which case their effect would be at a minimum. Death and desiccation would be accompanied by a concentration of these compounds, until finally they would be adsorbed by the cell walls and plasmatic colloids in their most concentrated condition with resulting coagulations, some of which in all probability are irreversible. The thin leaves have a higher acid content, and, to anticipate, a smaller proportion of pentosans which would accentuate this effect, hence the relatively low coefficient of swelling from a dried state. A long series of experiments with sections of dried colloids and of living and dried plants of known composition make it appear that the water relations of active tissues show the behavior of a biocolloid consisting largely of pentosans, of which agar or plant mucilages would be an example, a small proportion of protein or protein derivatives, and some salts and free acids.[4] It is to these features, therefore, that one would naturally turn for the factors which might increase the water-holding capacity of the cell or organ, and in so doing the pentosans would claim attention first. These substances probably are always present in some proportion in cells, and their occurrence is therefore not significant. Any action or condition which brings about a notable increase in their proportion in the cell would have most important consequences however. Such increase does result from a depletion of the water of a cell, for the polysaccharides under such conditions are reduced to the pentosans, and the reduction of the water content of a cell results in the conversion of the polysaccharides, which do not show marked imbibition, to pentosans, which take the form of an elastic gel with an enormous capacity for expansion, particularly when mixed with nitrogenous material, and upon this rests the hypertrophies or hyperplasias of thin-walled tracts in the development of

[4] MACDOUGAL, D. T., Imbibitional swelling of plants and colloidal mixtures. Science 44:502. 1916.

MACDOUGAL D. T., and SPOEHR, H. A., The effect of acids and salts on biocolloids. Science 45:269–272. 1917.

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succulence in an organ. Briefly restated, whenever the water content of a cell becomes low, some of the hexose-polysaccharides, which have a low imbibition capacity, are converted into pentosans, which have a high hydration capacity, the action having the force of a regulatory adjustment, and as the change is irreversible, the pentosans are accompanied by a permanent succulence, with all of the implied alterations in metabolism,[5] including a very striking change in the type of respiration, or of transformations in the carbohydrates.[6]

It is notable that, while this change in the sugars takes place in the cell, the type of transformations of energy changes completely, but the approximate rate of respiration is not materially affected. The nature and amount of the end products, however, may differ materially from those of a respiration in thin leaves, notably in the acid residues. It is in the mesh of reactions indicated that the origin and the nature of succulence will be found, and whatever causal value is attributed to the action of soil salts or of arid conditions will rest upon their part in the conversion of the polysaccharides to pentosans.

Acidity in succulents has been attributed by many writers, including the authors of this article, to the imperfect oxidations resulting from the lessened aëration of massive tissues, leaving a residue of malic acid, for example. *Castilleja*, however, presents the example of highly acid thin leaves, which become succulent under conditions similar to those which favor the transformation of polysaccharide to pentoses in other plants. Instead of acidity being a direct result of succulence, it is much more reasonable to conclude that high acid residues may be characteristic of plants which present a metabolic complex favorable to pentose formation and to the development of succulence under certain environic conditions.

[5] SPOEHR, H. A., The pentose sugars in plant metabolism. Plant World 20:365. 1918.

[6] MACDOUGAL, D. T., and SPOEHR, H. A., The origination of xerophytism. Plant World 21: 245–249. 1918.

COASTAL LABORATORY
CARMEL, CALIFORNIA

A CONIFEROUS SAND DUNE IN CAPE BRETON ISLAND

LeRoy H. Harvey

(WITH EIGHT FIGURES)

Nova Scotia has been called "the long wharf of Canada." Cape Breton Island, which is cut off from the mainland by the Gut of Canso, may be likened to its outermost pier. The island (fig. 1), which is about 100 miles long by 30 miles wide in its northern portion, extends in a northeasterly-southwesterly direction, restraining the waters of the Gulf of St. Lawrence on the west and separating them from the Atlantic Ocean on the east. The latitude of 47° north cuts the northern end of the island a few miles to the north of Aspy Bay, on whose shores the coniferous sand dune is located. Nova Scotia lies in the coniferous belt, which occupies the upland with the mixed hardwood formation occupying the most favorable situations along the narrow coastal strip. The interior is occupied by a vast expanse of wet and dry tundra-like formations, bordered by gnarled and twisted dwarf spruce, the entire vegetational aspect being decidedly coastal rather than alpine.[1]

The country is extremely rugged and the coastline jagged. Along the east coast is a narrow strip of sloping land, rarely a mile wide and often entirely lacking, which soon rises abruptly into an upland about 1000 ft. above sea level. In some places this upland plunges precipitously into the sea and the coast is very wild and bleak. This old Atlantic upland, which forms the backbone of the island, is the northern extension of the Piedmont Plateau. This upland has been cut during eons of erosion into deep gulches which extend far back into the central plateau. Down these gulches run swift and bowlder-bedded streams to the sea. At the mouth of these streams intervales are formed. Storm and tidal action have thrown shingle beaches and sand spits across the mouths of many of these reentrant bays (fig. 2). Upon one of these sand spits inclosing the South Pond of Aspy Bay is located the sand dune area which forms

[1] Nichols, G. E., The vegetation of Cape Breton Island, Nova Scotia. Trans. Conn. Acad. Sci. 22:251–467. 1918.

the subject of this study. It is the only sand dune noted on the island. The area is located some 5 miles from the crest of the upland and is fully exposed on its western side to the terrific northwest

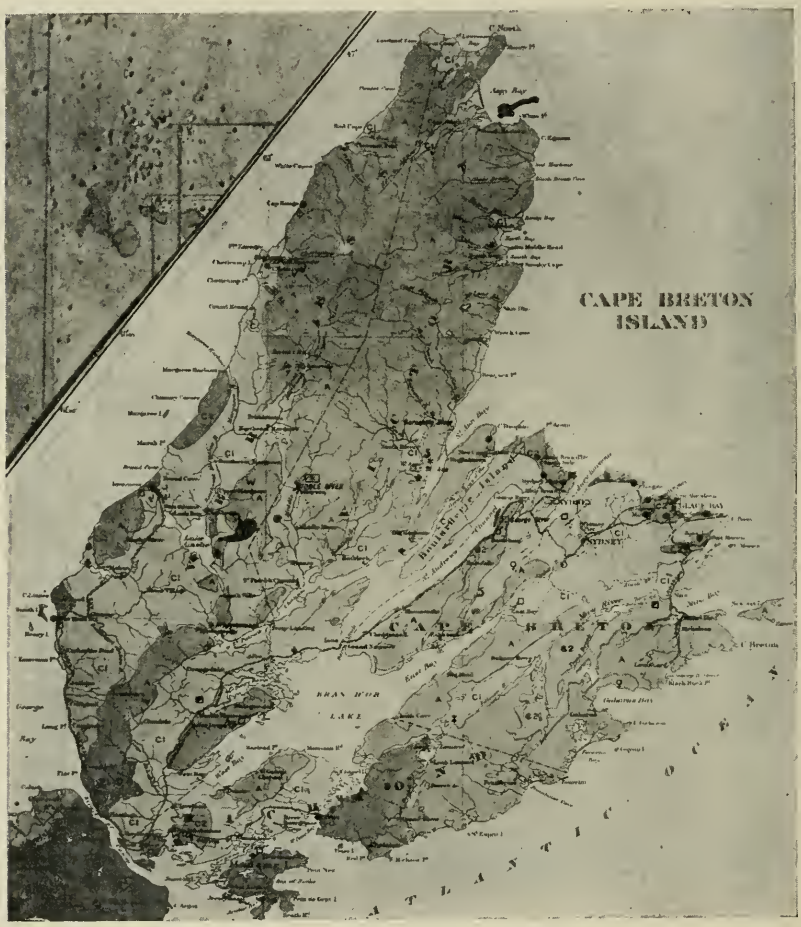

FIG. 1.—Cape Breton Island: Atlantic upland represented by dark shading; sloping marginal strip of lowland in light; Aspy Bay indicated by arrow.

winds, as well as to the cutting action of the outrunning tides from South Pond. The sand spit juts out to the southeast from a rocky upland reaching a total length of approximately $\frac{1}{2}$ mile, with a maximum width at the present time of less than 650 ft., and with a

minimum of less than 400 ft. But for the swift running tidal currents the spit would completely impound South Pond, extending across to the other headland; at best only a shallow and narrow channel now exists.

FIG. 2.—Aspy Bay region: sand spit on which sand dune is located marked by arrow; light shading indicates upland; dark shading indicates lowland.

The present condition of the area may be seen from fig. 3, which attempts to show the distribution of the existing plant associations. The dune complex, which is some 300 ft. wide, occupies less than one-half the length of the spit, being replaced in part at its southeastern extremity by a middle beach 400 ft. in width.

On the east the dune complex is fronted by a middle and lower beach, each with a width of approximately 100 ft. The lower

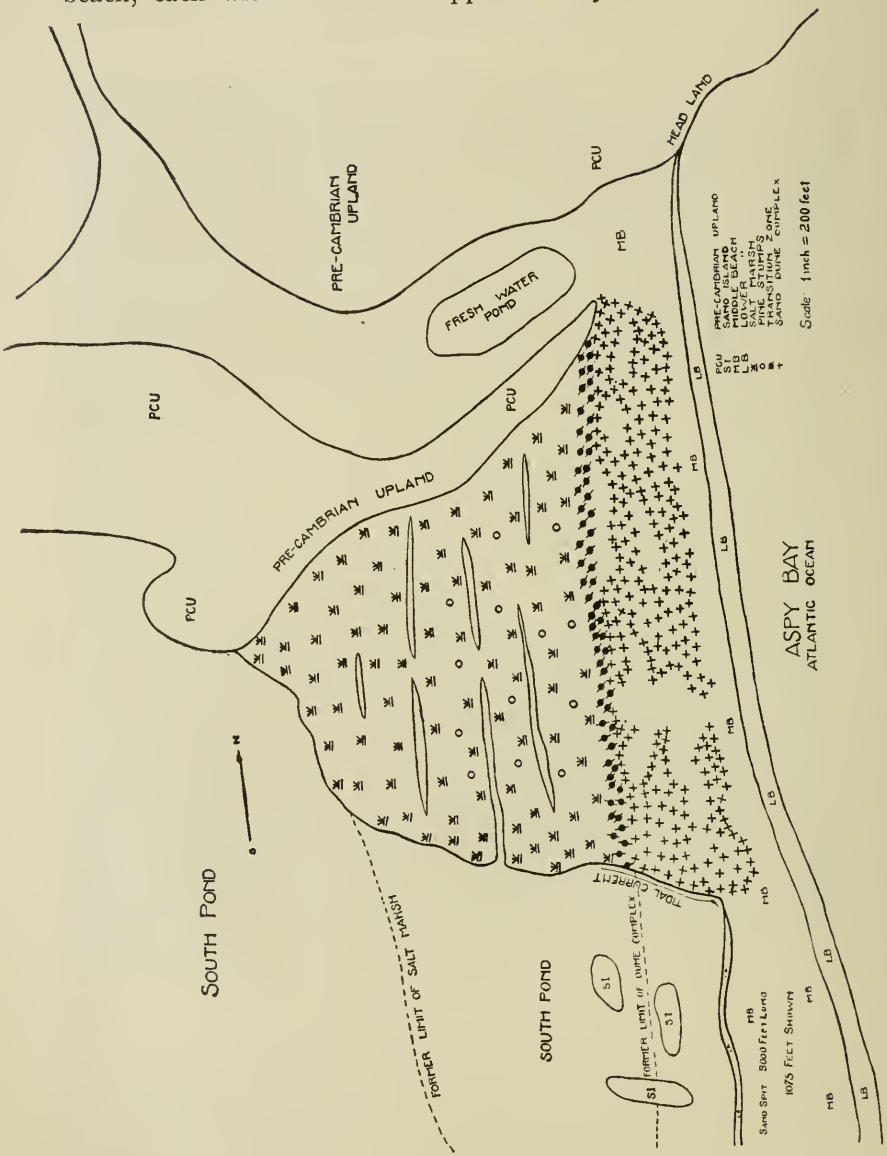

FIG. 3.—Diagram of present distribution of plant associations of dune complex

beach maintains its average width on the east side of the duneless end of the spit, where it has a gentle slope, but on its west side the

lower beach occupies a narrow steep margin only a few feet wide. To the immediate west of the spit in South Pond are several isolated sand islands (S.I.) mostly covered at high tide. To the west of the dune complex a transitional zone some 50 ft. wide separates it from an extensive salt marsh which is about a foot lower, and whose average width is estimated at 1200 ft. Several narrow salt water lagoons traverse this area in a north-south direction. Extending out into the marsh some 600 ft. along the eastern border is an area occupied by 10 or 15 old white pine stumps, approximately 100 years old, with well exposed roots, and standing in rows more or less parallel to the axis of the spit. The south end of the salt marsh and dune complex are suffering very active erosion under the daily outgoing tidal currents. High tides and occasional storms apparently sweep completely over the low duneless extremity of the spit, greatly augmenting this erosion. We may now consider each of these associations in greater detail.

Middle beach

The middle beach, which is extremely barren, is composed mostly of a fine sand, but shingle of a coarser nature is not wanting. The usual débris of the middle beach is encountered only to the east of the dune complex (fig. 4). The 3 principal plants are *Mertensia*[2] *maritima*, *Euphorbia polygonifolia*, and *Ammophila arenaria*, but all are exceedingly scattered. The only other species noted were *Glaux maritima*, *Lathyrus maritimus*, *Salsola Kali*, *Cakile edentula*, and these are represented only by occasional individuals.

Dune complex

The facies of the dune complex is *Picea canadensis* with occasional *Abies balsamea*. At the northern end the older trees were estimated at 75 years, while those at the southern, eroding end are scarcely 30–40 years of age. The southern (fig. 5) and eastern fringes of the complex include the highest dunes, which range from 3 to 15 ft. above sea level, and are generally margined by a narrow "grassy foredune" (fig. 6) with its precipitous slope oceanward. The sand binder is *Poa compressa*, a most unique condition. *Ammophila*, although scatteringly present, is of little importance in

[2] Nomenclature of GRAY's *New Manual*, 7th ed.

FIG. 4.—Southeast end of dune complex, from east, showing middle beach, the *Picea-Abies* stand, and grassy foredune; South Pond and highland beyond is shown at extreme left; photograph by Dr. G. E. NICHOLS.

FIG. 5.—Southern and eroding end of dune complex, from south: South Pond and highland to west seen at left; middle beach is well shown; evidence of recent and rapid erosion plainly evident.

this respect. Associated species are *Euphorbia polygonifolia*, *Taraxacum*, *Iris versicolor*, *Rhus Toxicodendron*, *Rubus* sp., and *Ribes oxycanthoides*, all of which occur sporadically.

On the thickly wooded lea slope of these grassy dunes are found numerous woody and herbaceous species. The most prominent

FIG. 6.—Grassy foredune held by *Poa compressa*, from north: middle beach and naked spit seen to south; highland shown to south of South Pond (barely visible); photograph by Dr. G. E. NICHOLS.

species are *Iris versicolor*, *Campanula rotundifolia*, *Vaccinium Vitis-Idea*, *V. pennsylvanicum*, *Maianthemum canadense*, *Rhus Toxicodendron*, *Ribes*, and *Rubus*. The occurrence of these forms is sporadic.

In some places this outer range of dunes passes toward the west into low areas of considerable extent occupied by a unique association (fig. 3, blank areas in dune complex). Its aspect is grassy,

determined mainly by *Festuca rubra*, *Danthonia spicata*, *Agrostis maritima*, and *Panicum implicatum* in rather open formation. *Lechea intermedia*, *Vaccinium Vitis-Idaea*, *Potentilla tridentata*, *Fragaria virginiana terra-novae*, *Juniperus horizontalis*, *Empetrum nigrum*, *Barbula*, and a species of moss form more or less extensive mats. Other more scattered species are *Campanula rotundifolia*, *Euphrasia americana* (?), *Cerastium arvense* (?), *Solidago bicolor*, *Plantago maritima*, *Iris setosa* (?), *Veronica serpyllifolia*, *Arenaria lateriflora*, *Plantago major*, and several ruderals.

FIG. 7.—*Picea canadensis* showing layering; individual trees plainly seen in center of fig. 5.

A second and in some places a third series of much lower dunes is met in transect to the west. At the northern end of the complex practically all the white spruce is excessively infected with *Arceuthobium pusillum*, presenting the most remarkable development of witches' brooms it has ever been my privilege to see.

We have here a most remarkable physiographic condition of a dune moving seaward. The trees have mostly germinated at a lower level, and as the sand blows over the rounded top of the "grassy foredune" it forms a gentle lee slope to the west among these trees. As the trees are covered, abundant layering takes place, giving a long-lived and self-perpetuating stand (fig. 7). There

is some evidence, however, that germination actually takes place on these grassy dunes. Through layering and germination the complex slowly moves oceanward.

Salt marsh

The aspect of the salt marsh is determined by *Spartina glabra*, *S. patens*, and *Distichlis spicata*. *Juncus balticus littoralis* is very abundant along the drier margins. Other common species are *Salicornia europaea*, *Potentilla pacifica*, *Ranunculus Cymbalaria;*

FIG. 8.—Salt marsh from eastern margin; *Pinus Strobus* stump in foreground; lagoons and South Pond in background; photograph by Dr. G. E. NICHOLS.

while *Vaucheria* and *Cladophora* occur in great mats on the margins of pools. The most striking feature, however, is the presence of numerous white pine stumps (fig. 8), remnants of a lumbering operation, whose distribution simulates rows parallel to the axis of the spit extending from the eastern shore out into the marsh to a distance of several hundred feet. The roots of these stumps are well exposed. It is evident that they must have germinated upon land possibly a foot or more higher than this, somewhat over a century ago. It is also evident that the salt marsh has encroached from the west and is moving eastward. Coastal elevation or denudation

could account for this encroachment, and I believe the latter more probable. The erosive force might well have been wind, acting subsequent to the removal of the white pine forest which evidently existed here. High tidal action has undoubtedly cooperated in the removal of the upper part of what appears to have been an extensive sand plain, as indicated by the north and south extensions of the lagoons.

Restoration of original condition

Within a century it seems probable that an area somewhat more extensive to the south than that now occupied by the salt marsh, and lying in the lee of the highlands to the north, was covered with a stand of *Pinus Strobus*. The present area of the dune complex, lacking this protection from marine influences, was covered with a stand of *Picea canadensis* and *Abies*, and this stand extended in its full width to the present end of the spit. It seems probable that the dune complex is a relatively recent phenomenon, developing subsequent to the removal of the stand of white pine. At about this same time the channel of the tidal current was changed and began cutting at the south end of the spit, eroding the *Picea* stand (fig. 5). According to a native, about one-third of this erosion has been accomplished in the last 35 years, or at the rate of 25 ft. per annum. If this rate has been approximately constant, the *Picea* stand was intact within a century to the end of the spit, which now lies bare for about one-half mile (fig. 3).

Summary

It is the purpose of this paper to put on record several facts of ecological interest: (1) a coniferous sand dune with *Picea canadensis* as its facies located at the latitude of 47° north; (2) *Poa compressa* as a sand binder; (3) abundant layering in *Picea canadensis* and *Abies balsamea;* (4) the anomalous condition of a sand dune moving seaward; (5) a phenomenal development of *Arceuthobium pusillum* on *Picea canadensis;* (6) the decisive value of ecological data in the interpretation of physiographic phenomena.

WESTERN STATE NORMAL SCHOOL
KALAMAZOO, MICH.

EMBRYO SAC AND EMBRYO OF PENTSTEMON SECUNDIFLORUS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 248

ARTHUR T. EVANS

(WITH PLATE XII)

Two genera of the Scrophulariaceae were the first plants in which the development of the embryo sac and the embryo were correctly investigated. In 1851 HOFMEISTER (8), working on *Lathraea squamaria* and *Pedicularis sylvatica*, proved that the embryo was formed as a result of the fertilization of the egg, and not from the end of the pollen tube as was believed by SCHLEIDEN and his followers. DEECKE (5) in 1855 reinvestigated *Pedicularis sylvatica*. He insisted that HOFMEISTER was wrong and that the embryo really did develop from the end of the pollen tube. He was supported in his assertions by SCHACHT (11). Later, however, HOFMEISTER (9) proved that what DEECKE really saw was the proembryo. In his paper on *Lathraea* and *Pedicularis*, HOFMEISTER discusses the beginning of the endosperm and the haustoria. No further work of importance was done upon the Scrophulariaceae until 1874, when CHATIN (3) studied the development of the ovule and the seeds in a number of genera. Four years later VESQUE (14) worked on the embryo sac of a number of families, among which were included several of the Scrophulariaceae. Even as late as his publication of this paper VESQUE believed that SCHLEIDEN'S theory of the formation of the embryo was correct, and criticized HOFMEISTER'S interpretation as inaccurate.

One of the best contributions to our knowledge of the embryo sac situation in this family is by BALICKA-IWANOWSKA (2) in 1899. The account includes a study of a number of families of the Sympetalae, but deals especially with several genera of the Scrophulariaceae, particularly taking up the question of nutrition in the embryo sac. The haustoria are believed to have an absorptive power, and thus conduct nourishment into the embryo sac, the

conclusions being based on the fact that the haustoria are always found in contact with parts which are well supplied with nourishment. Miss BALICKA-IWANOWSKA disagrees with HEGELMAIER (7) as to the function of the tapetum. HEGELMAIER believed it to act as a protective covering, while the former seemed to prove that it serves to pass nutritive substances on to the embryo sac, and that it possibly has a digestive function, since the cells of the integument adjoining it are found constantly breaking down. In 1906 SCHMID (12) investigated numerous species of the Scrophulariaceae. He discusses the formation of the embryo sac, fertilization, endosperm formation, and the development of the haustoria. He has done very little with the development of the embryo. In 1915 Miss MITCHELL (10) investigated the embryo sac and the embryo in *Striga lutea*, a semi-parasitic plant found in South Africa. In this form she has noted the lack of a tapetal layer.

The material for this study was collected near Boulder, Colorado, where the species is abundant. The indeterminate inflorescence affords flowers in all stages of development on the same plant. Such material was killed in chrom-acetic acid, cut in paraffin, and stained in various stains, safranin-gentian violet proving the best. Longitudinal sections 10 μ thick proved quite satisfactory for study.

The writer is indebted to Dr. FRANCIS RAMALEY of the University of Colorado for advice during the early stages of the work, and especially to Dr. CHARLES J. CHAMBERLAIN of this laboratory, under whose direction the work was completed, for his kindly aid and criticism.

Ovary and embryo sac

The ovary of *Pentstemon secundiflorus* Benth. is of the ordinary bilocular scrophulariaceous type, with the partition somewhat swollen in the median line forming the placenta, which bears the numerous crowded anatropous ovules. Longitudinal sections of such an ovary at right angles to the partition afford a large number of ovules in each section for study. Sections of very young ovaries show the ovules beginning as slight swellings of the placenta. The megaspore mother cell is not distinguishable in such an early stage, becoming apparent only after the beginning of integument forma-

tion. It appears as a single enlarged and darker staining subepidermal cell, which functions directly. Growth is quite rapid and the cell soon becomes elongated. It is surrounded by a single layered nucellus. The single integument forms along the sides of the nucellus and soon surrounds it. A short time before the integument has completely surrounded the ovule the megaspore mother-cell has entered synapsis (fig. 1). Miss MITCHELL has estimated that about 10 per cent of the ovules of *Striga lutea* have reached synapsis at the same time. This percentage may safely be placed much higher for *P. secundiflorus*, probably more than 75 per cent of the ovules of a single ovary showing the same stage of development.

By the time the nucleus of the megaspore mother-cell has entered synapsis the young ovule is rapidly assuming its anatropous form, which is reached by the time the reduction divisions are completed. The first reduction division occurs about the time the integument has surrounded the ovule completely. This division is soon followed by the second, forming the row of 4 megaspores. Either the third or the fourth megaspore of the row may function in forming the embryo sac (figs. 2, 3). The other 3 disintegrate rapidly and become crushed by the growth of the one functioning.

The megaspore which functions increases rapidly in size, the micropylar end becoming bulbous while the chalazal end remains narrowed (fig. 4). The chalazal end, however, lengthens rapidly until it is 2–4 times as long as the bulbous portion. This growth carries it to a point in contact with the end of the vascular system. The nucellus early disappears, but by the time the embryo sac is formed another nutritive layer, the tapetum, has formed from the integument. During the growth of the embryo sac the single nucleus by 3 divisions has formed the 8-nucleate sac. The rapid growth of the sac causes the protoplasm to be much vacuolated.

In the earliest stage of the 8-nucleate sac 4 nuclei are found grouped at each end (fig. 4). Soon, however, a nucleus from each end migrates toward the opposite end. Eventually they meet and form the polar fusion nucleus (fig. 5). BALICKA-IWANOWSKA and SCHMID have commented upon the place of this fusion. The former says that it occurs near the middle of the sac, while the

latter finds that it may occur anywhere in the sac of the Scrophulariaceae studied by him. In *P. secundiflorus* polar fusion was found to take place anywhere, seeming to be more a matter of chance than any regulated procedure. Regardless of where polar fusion takes place, the polar fusion nucleus is always found in the bulbous micropylar end of the sac at the time of fertilization (figs. 6, 7). It is here that the triple fusion is completed. By the time polar fusion is completed the egg apparatus is well formed and the antipodals have begun to disintegrate. In only one case were the antipodals observed to form anything resembling cell walls. The mature embryo sac (fig. 5) is one of the commonest stages of the sac to be found. This is probably due to failure to pollinate at once. A short period of inactivity always seems to occur.

The mature embryo sac is interesting in that it is always well filled with starch (figs. 6, 7). As soon as the megaspore begins its development into the embryo sac, traces of starch are to be found in it, although it is not until the embryo sac is well matured that large quantities of starch are present. Very often the adjacent tissues contain much starch also. After fertilization, when the endosperm and the embryo begin to develop, the starch in the sac disappears entirely. Many of the grains found in the sac are large, reaching 30 μ or more. Although starch is to be found in either end of the embryo sac it is always much more abundant in the micropylar end. SCHMID has found this to be true also.

False polyembryony

The fusion of 2 ovules appears to be a much more uncommon occurrence than the formation of 2 or more embryo sacs in a single ovule. Miss MITCHELL discusses a single case which she found in *Striga lutea*. The only other plants in which it has been reported are *Pyrus Malus*, *Loranthus europaeus*, and *Viscum album* (4). In the course of this study several cases in which 2 ovules had fused were noted. In some the fusion was quite complete, in others the ovule could be seen to be double. The presence of 2 micropyles as well as integumentary tissue between the 2 embryo sacs indicated that 2 ovules had fused. In one instance noted the

egg apparatus had formed and polar fusion had occurred in both embryo sacs. False polyembryony seems to be quite common in this species.

Fertilization

In several cases the pollen tube with the tube nucleus and the 2 sperms were observed. While in the pollen tube the sperms are more or less capsule-shaped, but after reaching the embryo sac they become quite spherical. The pollen tube seems always to enter the embryo sac a little to one side, its entrance usually destroying one of the synergids, the other synergid disappearing soon afterward.

The sperms are readily distinguished from the egg nucleus and the polar fusion nucleus on account of their much smaller size. Fertilization of the egg and the triple fusion always occur in the micropylar end of the sac and in a normal manner. Both fusions occur at approximately the same time.

Several cases of double fertilization were observed (fig. 7). Previously double fertilization has been announced as occurring in *Digitalis purpurea*, *Linaria vulgaris*, *Melampyrum sylvaticum*, *Lathraea squamaria*, *Pedicularis foliosa*, and *Striga lutea* of the Scrophulariaceae. This adds *Pentstemon secundiflorus* to the list.

Formation of endosperm

Without resting after the fusion with the sperm, the endosperm nucleus by a series of divisions forms a large number of nuclei, which migrate to the chalazal end of the sac and there become peripherally placed. Simultaneous with the formation of the free endosperm nuclei the narrowed end of the sac begins to increase in size very rapidly, so that it soon surpasses the micropylar end in diameter (fig. 8). By the time the first endosperm walls have formed this end of the sac is much the larger. During all this increase in size a certain restricted area between the 2 ends remains very narrow, so that the embryo sac comes to be dumb-bell-shaped, with the chalazal end the larger. Endosperm walls continue to form in this end until the whole is completely filled (figs. 8, 10). Although endosperm nuclei are occasionally found in the micropylar end of the sac, no cell walls were observed to form. During

endosperm formation the tapetum appears to be very active. Integumentary cells in contact with it are broken down and the tapetal cells are always filled with a dense protoplasm.

Haustoria

With the formation of the endosperm 2 large haustoria are formed: one in the neck which connects the micropylar and chalazal ends of the sac (fig. 8), the other as an outgrowth from the chalazal end of the sac (fig. 9). The former is formed by the growth of 2 endosperm cells forward through the narrowed neck and just into the micropylar end of the sac where growth stops. In the case of the chalazal haustorium there is an outgrowth of the sac in the region not covered by the tapetum. Into this bulbous pocket 4 endosperm cells grow. This brings the endosperm cells well into connection with the vascular tissue, the cells of which are gorged with nutritive material. The protoplasm of each haustorium is very dense. The cells of the chalazal haustorium are binucleate.

The active tapetal layer covers only the chalazal end of the sac (fig. 8), ending abruptly at its junction with the micropylar end. SCHMID found that the tapetum might cover all of the embryo sac or only part of it as in *P. secundiflorus*. The latter condition seems the more common occurrence. Miss MITCHELL found that no tapetum is formed in *Striga lutea*. She believes that this may be accounted for by the semi-parasitic habit of the plant.

Development of embryo

After fertilization the egg rests for a time, often even until endosperm cell walls have begun to form. It then divides, the first division being at right angles to the axis of the embryo sac. The segment nearest to the micropyle forms the suspensor, the other forming the embryo. By a series of divisions, coupled with rapid growth, the suspensor is transformed into a bulbous basal portion, and a number of smaller narrowed cells which lengthen rapidly in such a manner as to push the 1-celled embryo through the micropylar end of the sac (fig. 8) and into the center of the endosperm beyond (fig. 10). It is usually pushed from one-third to one-half

of the way through the endosperm, where further progress is probably stopped by the division and growth of the embryo. The first 2 divisions of the embryo are at right angles to each other and in the plane of the long axis of the sac. The next division is at right angles to the first two and forms the 8-celled stage of the embryo. The 16-celled stage is formed by the periclinal division of the cells of the octant. The further division of the embryo was not followed.

After the embryo becomes imbedded in the endosperm the micropylar end of the sac, together with the suspensor, collapse and disappear. Their disappearance is accounted for by the pressure within the ovule, due to the increase in amount of endosperm which eventually comes to occupy all the space inside the seed coat.

Discussion

In the formation of the embryo sac of *P. secundiflorus* there is nothing strikingly different from that of other species of this family which have been studied, but the shape of the mature embryo sac is peculiar. The very bulbous micropylar end, with the long, narrowed chalazal end, gives the whole embryo sac a club-shaped appearance. The chalazal part of the embryo sac is never more than half as wide as the micropylar end at the time of fertilization. The drawings of the embryo sac of other Scrophulariaceae by Balicka-Iwanowska, Schmid, and Mitchell show that there is a tendency toward this shape of embryo sac in the family, but none of those drawn are so striking in shape as that of the species under consideration. The distance between the end of the embryo sac and the end of the vascular system is at first marked. As the sac later derives a large part of its nourishment through the vascular system, this may account for the necessity of lengthening the sac until the end comes in contact with this source of food supply.

During the development of the embryo sac traces of starch can be seen within it, and in all cases, by the time fertilization occurs, large quantities of starch are present. Often it is so abundant that the nuclei within the sac are partially or entirely obscured. By the time the embryo has reached the endosperm the starch has all disappeared.

D'HUBERT (6) has made a study of fleshy plants with regard to the formation of starch in the embryo sac. He finds that starch is always present in the sacs of fleshy plants such as the Cactaceae, Mesembrianthaceae, Crassulaceae, Portulacaceae, etc. He has also found, however, that some non-fleshy plants show starch in the embryo sac. According to D'HUBERT this latter case seems to be the exception rather than the rule, and he believes that there is a relationship between the fleshiness of the plant and starch in the embryo sac due to the slowness of the phenomena before fertilization. This, however, receives very little attention from him; nevertheless it seems the more plausible theory. *P. secundiflorus* is not a fleshy plant, but, judging from the drawings which D'HUBERT has made of several fleshy plants, it has more starch in its embryo sac than any of those figured. It appears that while there is activity in the embryo sac very little if any starch is stored up. As soon as the embryo sac matures and becomes inactive just before fertilization, possibly due to delay in pollination, the stream of nourishment which has been coming in cannot be checked suddenly but keeps passing more and more nutrition into the inactive sac, where it is stored in the form of starch. Such a conclusion seems to be substantiated by the fact that activity in the sac brought about by fertilization soon reduces the amount of stored-up starch. BALICKA-IWANOWSKA (2) has also investigated the deposition of starch in the embryo sacs of several plants, and has concluded that starch is only found in the embryo sac when the tapetum is cutinized. This does not seem to be the case in *P. secundiflorus*, however, as the tapetum is undoubtedly not cutinized. Moreover, it covers only half of the embryo sac, as has been explained before. SCHMID (12) has found starch present in the integuments as well as the embryo sacs of a number of the Scrophulariaceae. He states that the starch is found throughout the embryo sac, but that sooner or later it is all translocated to the micropylar end, "wo die lebhaftesten Teilungen stattfinden."

The function of the tapetum seems to be one of nutrition, as has been suggested by BALICKA-IWANOWSKA (2). That it may have a protective function, as has been suggested by HEGELMAIER (7), seems rather doubtful. This seems all the more questionable

when one considers that it covers only the chalazal end of the sac in a number of species. Surely the micropylar end would be as much in need of protection. In *P. secundiflorus* the integumentary cells border on the micropylar end of the sac.

The two haustoria function in passing nourishment to the endosperm cells which are farther from the supply of food. By the time the embryo has reached the endosperm the micropylar haustorium becomes inactive and is lost. The chalazal one, however, functions until the endosperm is formed. The nuclei in this haustorium are very pronounced. On account of the large size and seeming activity of haustorial nuclei some authors have attributed to them a considerable rôle in nutrition. BALICKA-IWANOWSKA (2) has always found them near the point where nutrition is most abundant. In this work a similar tendency was noted.

The growth of the suspensor in such a manner as to push the proembryo through the micropylar end of the embryo sac and to imbed it in the endosperm is rather unique. SHARP (13) in his study on *Physostegia* has recorded a similar situation, but the method of endosperm formation in *Physostegia* and *Pentstemon* is different entirely.

During the growth of the suspensor which imbeds the embryo in the endosperm, nutrition is derived from the starch stored up in the micropylar end of the sac.

Summary

1. The embryo sac is developed from a single megaspore. Its antipodals disorganize early. The micropylar end becomes bulbous, while the chalazal end becomes long and narrow and is covered by a distinct tapetum.

2. The mature embryo sac is found to be constantly gorged with starch, due to the non-utilization of the nutritive materials which pass into the sac at a time of inactivity just before fertilization.

3. The endosperm nucleus immediately divides and free nuclei migrate into the chalazal end of the sac, where wall formation begins. The proembryo is pushed into this endosperm by an

extreme growth of the suspensor. The micropylar end of the sac disintegrates.

4. Two haustoria are formed, the micropylar by the growth of endosperm cells from the chalazal end into the micropylar end, and the chalazal by a growth of endosperm cells from the chalazal end out into the vascular system. The cells of the latter haustorium are binucleate.

5. False polyembryony occurs rather commonly in this species.

PURDUE AGRICULTURAL EXPERIMENT STATION
LAFAYETTE, IND.

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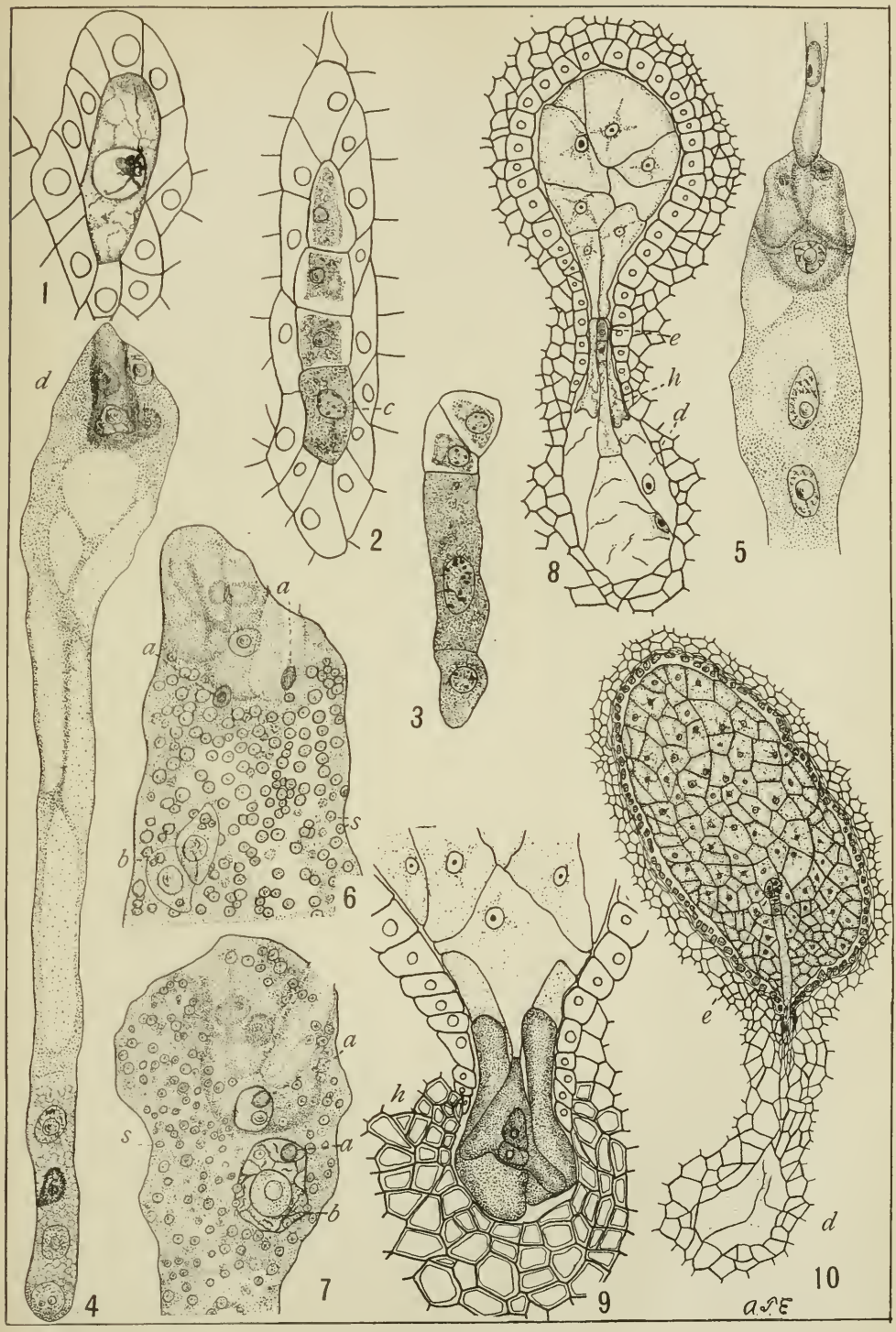

EVANS on PENTSTEMON

EXPLANATION OF PLATE XII

All the drawings were made at table level with the aid of an Abbé camera lucida. The labels are: *a*, sperm; *b*, polar fusion nucleus; *c*, megaspore; *d*, micropylar end of embryo sac; *e*, embryo; *h*, haustorium; *s*, starch; *t*, tapetum.

FIG. 1.—Synapsis in the megaspore mother-cell; ×880.

FIG. 2.—Row of 4 megaspores showing fourth developing into embryo sac; ×880.

FIG. 3.—Row of 4 megaspores showing third developing into embryo sac; ×880.

FIG. 4.—An 8-nucleate embryo sac before maturity, showing characteristic shape of sac and egg apparatus beginning to form; ×880.

FIG. 5.—Micropylar end of mature embryo sac showing egg apparatus well formed and migration of polar fusion nuclei; ×460.

FIG. 6.—Micropylar end of mature embryo sac showing egg apparatus, entrance of pollen tube, 2 sperms, polar fusion, and starch; ×880.

FIG. 7.—Mature embryo sac showing double fertilization; ×880.

FIG. 8.—Stage showing growth of suspensor pushing young embryo between micropylar haustoria which have already begun to disintegrate; section a little to one side of chalazal haustorium; tapetum present; ×460.

FIG. 9.—Stage showing binucleate chalazal haustorium; ×880.

FIG. 10.—Late stage of endosperm formation showing embryo imbedded; micropylar end of sac already beginning to collapse; ×460.

BRIEFER ARTICLES

DEPRESSED SEGMENTS OF OAK STEMS

(WITH FOUR FIGURES)

In a recent paper, Miss LANGDON[1] questions certain statements of the writer[2] in regard to the deeply depressed or sunken segments which occur commonly in stems of *Quercus*. She states (p. 321):

> From observations of transverse sections of twigs from *Quercus alba*, *Q. bicolor*, and *Q. macrocarpa* I find that there is evidence of retardation in growth of the tissues in the immediate vicinity of the wide rays, especially noticeable in the marked dipping in of the annual rings where they cross the large rays. However, aside from a few extreme cases, this checking influence of the wide foliar rays does not explain the 5 conspicuous depressions so characteristic of the wood of *Quercus*.

In discussing the topographical features of the stem of the oak, it is essential to distinguish between two different factors which have modifying effects upon the outline of the secondary xylem. I refer to the arrangement of the primary elements and the development of multiseriate rays. The effects of these two factors, or complexes of factors, may be studied most satisfactorily in plants where they occur independently. For example, in *Castanea dentata* and *Populus balsamifera*, which normally have only uniseriate rays, the primary elements have the stellate arrangement that is characteristic of *Quercus*. In the internodes of normal stems of these plants, the first formed secondary elements form a layer of undulating or stellate outline, but at the end of two or three growing seasons, frequently earlier, the outer periphery of the secondary xylem tends to be circular (fig. 1). In other words, the early lobed or stellate form of the cambium soon becomes evanescent, and its effects upon the shape of the stele are quite transient.

The modifying influences of the second set of factors, acting independently of the first, may be seen quite clearly in the stems of certain

[1] LANGDON, LADEMA M., The ray system of *Quercus alba*. BOT. GAZ. 65: 313–323. 1918.

[2] BAILEY, I. W., The relation of the leaf trace to the formation of compound rays in the lower dicotyledons. Ann. Botany 25:225–241. 1911.

species of *Amphilophium*. Fig. 2 illustrates a cross-section of the stem of one of these plants. There are 4 pairs of approximated multiseriate rays in the fourth growth layer. The narrow segments of xylem between these wide rays are deeply depressed below the general outline of the stem. They obviously are not correlated with a lobed or stellate arrangement of the primary elements, but are due to differences in the number of xylem elements formed by different arcs of the cambium during the fourth growing season.

That the deeply depressed segments which occur commonly in oak stems, having 2–10 or more growth layers, are correlated with the presence of pairs of approximated multiseriate rays rather than the

FIG. 1

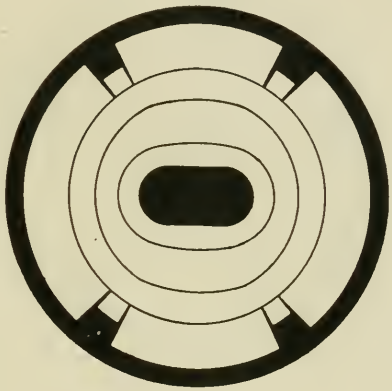

FIG. 2

stellate arrangement of the primary elements, is indicated not only by a comparative study of the stems of various arborescent dicotyledons, but also by numerous facts in the anatomy of the genus *Quercus*. From different species of oaks and from plants grown under different environmental or experimental conditions, it is possible to secure a series of stems showing various stages in the disintegration and disappearance of multiseriate rays.[3] The segments are most deeply depressed in specimens in which the pairs of multiseriate rays are most conspicuously developed (fig. 3). On the other hand, where the pairs of wide rays or their vestiges ("aggregate rays," etc.) are entirely absent, the stellate form of the early cambium, which may be conspicuous during the first growing season or two, quickly becomes circular, as in *Castanea* and *Populus*. Where there

[3] BAILEY, I. W., and SINNOTT, E. W., Anatomical evidences of reduction in certain of the Amentiferae. BOT. GAZ. **58**:36–60. 1914.

is a marked retardation in the development of the pairs of rays the appearance of the deeply depressed segments is coincident with that of the rays (fig. 4). Particularly significant are those stems in which one ray of a pair fails to develop. Under these circumstances, the narrow segment of xylem tends to be unsymmetrically depressed (fig. 4). Furthermore, the fact that depressed segments may occur between pairs of rays, which are opposite the projecting lobes of the pith (fig. 3), and between approximated "secondary" rays, suggests that the stellate outline of the early cambium is not an indispensible factor in the production of the sunken wedges of xylem in oak stems.

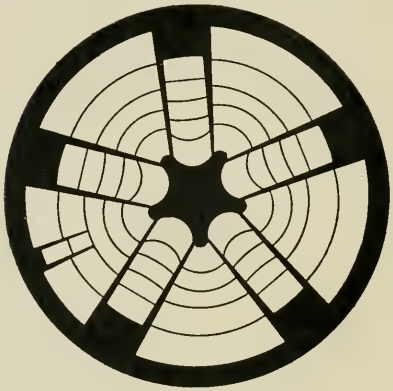

FIG. 3

FIG. 4

Miss LANGDON offers a physiological explanation for the stellate form of the stele in young twigs (p. 321):

> Since the principal function of the xylem is the conduction of water from the soil to the outer parts of the plant, it is obvious that the maximum upward movement of solutions in the stem would be through the tracheidal tissues and vessels in direct line with the leaf traces. This would cause an acceleration in growth and the consequent outward projection of those five regions of the woody cylinder associated with leaf traces, while the neighboring conducting tissues, namely, the so-called depressions from which the main conducting streams had been diverted to the petioles of the leaves, would fail to maintain their normal rate of growth.

It is to be emphasized, in this connection, that the projecting wedges of the first annual rings of *Castanea*, *Populus*, and *Quercus*, when devoid of wide rays, are not due to an acceleration of growth. This can readily be determined by measuring the depth of the convex and concave arcs of

xylem in one or two year old stems. The average depth of the latter almost always equals and usually exceeds that of the former (fig. 1), indicating conclusively that there is no growth acceleration in the convex arcs of the cambium which form the projecting wedges. As has been indicated by the writer, the undulating outline of the first formed secondary xylem is due to the stellate arrangement of the primary elements, and consequently the stellate outline of the first formed cambium. However, this originally lobed cambium rapidly takes on a circular outline, owing to the slower growth of its convex projecting arcs, except in stems which have a hereditary tendency for the formation of pairs of approximated multiseriate rays.—I. W. Bailey, *Bussey Institution, Jamaica Plain, Mass.*

IMPORTANCE OF EPIDERMAL COVERINGS[1]

(WITH TWO FIGURES)

In making tests of the relative resistance of some herbaceous plants to freezing, it was observed that inoculation from ice formed on the leaf surface was a factor of great importance in determining the temperature at which ice formation occurred in the leaf tissue. In testing cabbages it was observed that the greatest undercooling of the tissue below its freezing point occurred in those plants which had the greatest amount of "bloom" on the leaf surface. Plants well covered by wax could be maintained for hours at a temperature 5°C. below their freezing point without the formation of ice in the tissues. Similar conditions were found to occur in the common *Cineraria* and other such plants which are densely covered with a mat of epidermal hairs. This condition suggested that inoculation of the undercooled leaf tissue by ice formed on the leaf surface was an important factor in frost resistance. The object of this study was to determine the amount of undercooling which can occur in such tissues, and the importance of the epidermal coverings in preventing surface inoculation of the undercooled tissues.

The thermoelectric method was used to measure temperatures, since this method allows one to determine the temperature inside rather thin leaves. A copper-constantan couple of no. 40 B. and S. gauge which had a thermal coefficient of 3.33 millivolts per degree Centigrade was used. Using such a couple the delicacy of the potentiometer arrangement determines the accuracy of the temperature measurement. Although with the arrangement used much smaller changes could be

[1] Published by permission of the Secretary of Agriculture.

determined, measurements to 0.1°C. or less were found to be sufficiently accurate for this work.

Fig. 1 shows the arrangement for temperature measurement as well as the means for securing undercooling of the tissues. Directions for constructing the thermal junctions are to be found in the publications of WHITE from the Carnegie Institution of Washington.[2] The constant temperature junction of the thermocouple was placed in a thin-walled tube filled with oil and immersed in a slush of clean snow in distilled water contained in a Dewar beaker. It was found that the temperature of this junction could be kept constant for a long time within a a few thousandths of a degree Centigrade, as shown by a standard Beckmann thermometer. In calibrating the couple, the junction to be placed within the leaf was placed in a Dewar beaker similar to the former arrangement, with solutions having freezing points a few degrees below zero.

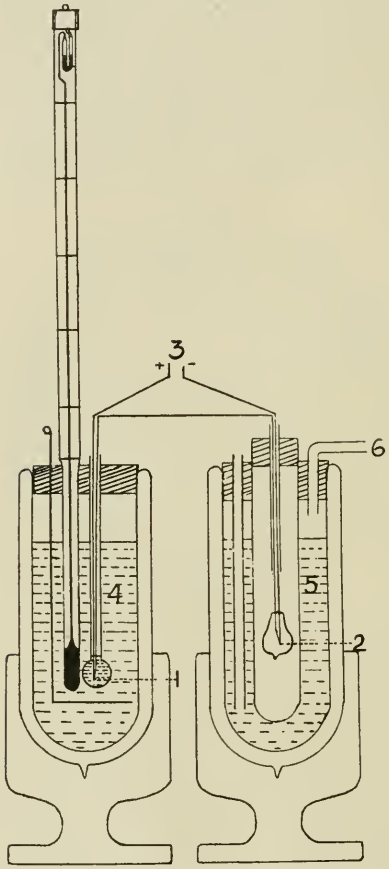

FIG. 1

A number of tissues were tested with this apparatus, including carnation stem, cabbage leaf, *Echeveria* leaves, *Cineraria* petiole, tomato petiole, and others. In general the results show that plants with heavy epidermal coverings of wax or trichomes can undergo a much greater undercooling than such plants as tomatoes, in which the epidermal walls are thin and non-resistant.

[2] WHITE, W. P., The thermoelement as a precision thermometer. Physical Review 31:135. 1910.

———, The constancy of thermoelements. Physical Review 23:449. 1906.

WHITE, W. P., DICKINSON, H. C., and MUELLER, E. I. The calibration of copper-constantan thermoelements. Physical Review 31:159. 1910.

To make certain that all of the samples should have ice formed on the surface, a small drop of water was placed on each. To test the effect of the epidermal covering in preventing inoculation, samples of the same material were cooled with the coverings intact, and also after they had been removed. Precautions were taken to prevent the inoculation of the tissue from juices exuded at cut surfaces. In all cases the cut surfaces were dried and covered with vaseline, which procedure was found to prevent such an inoculation of the tissue. In all the tests it was

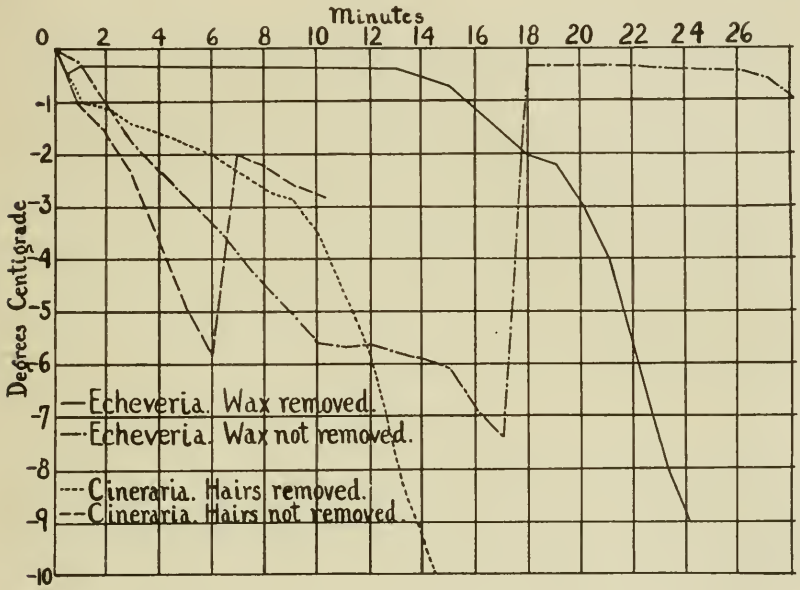

FIG. 2

found that when thick epidermal coverings were unbroken, undercooling occurred, while in those cases where they were removed but little undercooling occurred.

Fig. 2 shows typical cooling curves, indicating the importance of the epidermal coverings in preventing ice formation within the tissue. It appears that the tissue without the protective covering is unable to undergo any great amount of undercooling. This depends in part upon the amount of shaking of the tissue. The osmotic concentration or the presence of colloidal substances in solution in the cell sap are of relatively minor importance in determining the undercooling.

It has been observed during spring frosts that in blooming apricots those buds which have opened and are turned upward to collect snow are frozen, while those turned downward may not be injured. WEST and EDLEFSEN[3] attempted to prevent frost injury to apricot buds by spraying the trees with water. Instead of the desired effect of preventing freezing, this procedure evidently killed the tissue by allowing inoculation from ice formed on the surface. Trees which were not sprayed with water were not injured, although subjected to the same temperature. These examples serve to illustrate the importance of surface inoculation in producing frost injury.

The amount of undercooling in plants is not generally very great, nor is it sufficient to account for true frost hardiness. Such herbaceous plants as cabbage, kale, turnips, however, which ordinarily can withstand a considerable degree of freezing, acquire hardiness quite rapidly. In the case of cabbage, a temperature of 3°C. was found to harden the plants sufficiently in 5 days to allow them to be frozen stiff at −3°C. without injury. The principal importance, therefore, of the epidermal coverings for the frost resistance of such plants appears to be that they allow the plants which possess them to withstand temperatures somewhat below zero until the cells are able to adapt themselves physiologically to the changes incident upon freezing.

Summary

Undercooling of the tissues occurs to a greater degree in such herbaceous plants as possess protective epidermal coverings than in plants not so protected. The undercooling in such plants is not due to substances in the cell sap, but mainly to the prevention of inoculation from ice formed on the surface of the tissue. A method is given for determining electrically the temperatures within leaf tissues.—R. B. HARVEY, *Bureau of Plant Industry, Department of Agriculture, Washington, D.C.*

[3] WEST, F. S., and EDLEFSEN, N. E., Orchard heating. Bull. 161, Utah Agric. Coll. Exp. Sta. 1917.

CURRENT LITERATURE

NOTES FOR STUDENTS

Conditions affecting flower development.—KLEBS[1] divides the process of flower formation by the rosettes of *Sempervivum Funkii* and *S. albidum* into 3 distinct, successive steps: (1) production of the condition of ripeness to flower (blühreife Zustand), (2) formation of flower primordia, and (3) development of flower clusters and elongation of the axis. Light is the dominant factor in determining all 3 of these stages of development.

In the first and third, light is effective entirely through its photosynthetic action, and its effectiveness rises with its energy value. Higher temperatures counteract light by favoring dissimilation. Accordingly, the effect of temperatures can in part be annulled by increased light intensities. It is the balance of assimilation over dissimilation that furthers the development of these 2 stages. KLEBS finds that at lower temperatures (about 6°C.) both these stages can be attained in darkness, although in the last it gives a far less extensive inflorescence. He thinks this is likewise tied up with a balance in favor of available carbon synthate. The low temperature gives low respiration and leads to the accumulation of soluble sugars by the hydrolysis of insoluble carbohydrates.

In the second step, formation of flower primordia, light has 2 distinct and antagonistic effects. The one which favors the process is due to the photosynthetic activity of the light and is a function of the less refrangible rays of the spectrum. The other, which inhibits the process or even annuls the ripe to flower condition, must at present be termed a stimulus effect, and it is a function of the less refrangible blue rays. Diffuse daylight is relatively injurious to primordia development because of the high percentage of blue violet rays it contains. The Osram light and direct sunlight favor this development because of the dominance of the red rays.

KLEBS says it is still an unanswered question whether inflorescence development in other forms and in plants in general can be divided into these 3 distinct steps with similar light effects in each step. He suggests some facts as evidence that such may be the case. His past work has done much to show that the formative effects of conditions on plants is largely through the nutrient effects of these conditions. Thus the formative effect of light is explained in a large part by its effect on carbon assimilation, but KLEBS points out here, as in his

[1] KLEBS, GEORGE, Über die Blütenbildung von *Sempervivum*. Festschrift zum ERNST STAHL. pp. 128–151. Jena. 1918.

older work, that there is also a specific formative action of the blue rays as yet unexplainable on the nutrient basis.

He has often distinguished between the amount of carbon synthate and the amount of salt nutrients as formative factors in the plant, especially in connection with reproduction; and now FISCHER[2] makes this more definite by considering the nitrogen supply as the most important formative factor furnished by the salts, and by speaking of the carbon nitrogen ratio (C/N) of plants. He probably would not deny that the supply of other nutrient elements, phosphorus, calcium, potassium, etc., have at least minor formative effects and often of an opposite nature from nitrogen. This ratio can be increased by increasing the photosynthesis of the plants or by decreasing the nitrogen supply. The ratio can be decreased by decreasing photosynthesis or by increasing the nitrogen supply. FISCHER comes to this important conclusion. Very high C/N in plants favors flowering, while a low C/N favors vegetation. His conclusions are largely based on his own work on the effect of increased partial pressure of carbon dioxide upon the development of plants, but not upon chemical analysis of the tissues.

KRAUS and KRAYBILL[3] have recently worked upon the tomato, varying the C/N in it by varying its nitrogen supply. On the basis of extensive cultures and chemical, microchemical, and anatomical studies, they come to the following conclusions: (1) a very high C/N gives little vegetative growth and poor reproduction with a high percentage of dry matter; (2) medium C/N gives moderate vegetation growth, good reproduction, and a medium percentage of dry matter; (3) very low C/N gives very vigorous vegetative growth, little reproduction, and a low percentage of dry matter. KRAUS's extensive horticultural investigations enable him to give much evidence that the C/N ratio is a factor of great significance in determining fruitfulness in many economic plants. The contribution apparently puts into the hands of producers one of the important means of controlling fruitfulness. FISCHER's less extensive and one-sided attack caused him to miss the fact that a very high C/N not only reduces vegetative growth but diminishes reproduction.

These papers have thrown much light on some of the nutrient factors modifying vegetation and reproduction in plants.—WM. CROCKER.

Loss of chlorophyll.—MEYER[4] notes that in *Tropaeolum majus*, growing in pots in a greenhouse, the young leaves at the top of the stem are dark green, while the progressively older ones down the stem are green, bright green, yellow

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[2] FISCHER, H., Zur Frage der Kohlensaure-Einahrung der Pflanzen. Gartenflora 65: 232–237. 1916.

[3] KRAUS, E. J., and KRAYBILL H. R., Vegetation and reproduction with special reference to the tomato. Oreg. Agric. Exper. Sta. Bull. 149. pp. 90. 1918.

[4] MEYER, ARTHUR, Eiweiszstoffwechsel und Vergilben der Laubblätter von *Tropaeolum majus*. Festschrift zum ERNST STAHL. pp. 85–127. Jena. 1918.

green, yellow and bright yellow, and finally the oldest ones on the plant are wilting. MEYER points out that this change in color is due to the gradual decomposition of the chlorophylls, while the carotin and xanthophyll remain constant. As this change progresses the chloroplasts become smaller, and in later stages are shriveled granular masses with balls of excreted material about them. With the gradual loss of chlorophyll goes a similar decomposition of the proteins of the chloroplast. It should be mentioned that MEYER adduces evidence for the view that the chloroplast is the main organ for the storage of the proteins manufactured in the foliage leaf, if indeed not the very seat of protein manufacture. The amount of carbohydrates in the leaves also falls with age. MEYER found that when leaves are placed in darkness no reduction occurs in the proteins until the carbohydrates are greatly reduced by respiration. The decomposition of the proteins then begins, he believes, as a source of carbon chains for respiration. He states that there is no loss of nitrogen from the leaf during this change, but that the nitrogen residue remains in the leaf, while the carbon chain of the protein is used for respiration. He apparently gives the following interpretation of the process: As the leaves become older they become weakened; in this weakened condition the photosynthetic power falls; this leads to a great reduction in the amount of carbohydrates in the leaf, and finally to the decomposition of the proteins of the chloroplasts as a carbon source for respiration; this decomposition of the proteins is accompanied by the decomposition of the chlorophyll and the change in color.

SCHERTZ, in an unpublished work from this laboratory, finds in many respects parallel behavior in *Coleus Blumei*. He finds that shortage of nitrates leads to the decomposition of the chlorophyll, and that old leaves can be maintained green by addition of nitrogen fertilizer. He also finds the phospholipine content of the leaf greatly reduced as yellowing progresses. His evidence seems good that shortage of nitrogen initiates all of the decomposition of nitrogen compounds (chlorophyll, phospholipines, and proteins), and that it must be looked at as the immediate cause of the loss of chlorophyll. Plants grown in pots are likely to become pot bound and limited in their supply of soil nutrients.

There are many incompletely worked phases in MEYER'S paper; he has filled in some gaps by drawing data from other workers on very different materials; and his work leaves much to be desired in quantitative determinations and cultural experiments. All these leave interpretation to bridge broad chasms, and it is therefore not strange if he has missed the initiating cause of loss of chlorophyll.

If SCHERTZ is right, that the decomposition of chlorophyll in *Coleus Blumei* is due to shortage of nitrogen as a building material, it is also conceivable that a great excess of nitrogen may sometimes lead to the decomposition of chlorophyll due to the dearth of carbon chains produced by the excess of nitrogen. Shortage of magnesium as a building material may sometimes act in a similar way.—WM. CROCKER.

Knop's solution.—TOOLE and TOTTINGHAM[5] find that additions of $Fe(OH)_3$ to Knop's solution greatly increases the growth of barley tops in it (21 day cultures), while additions of carbon black depress the growth of tops and additions of H_2SiO_3 have no effect. None of these additions affect the growth of the roots. Part of the beneficial action of the $Fe(OH)_3$ may be due to its neutralizing action on the acids of the solution. It is interesting to note that the higher additions of $Fe(OH)_3$ removed 90 per cent of the phosphorus from solution.

In another piece of work, TOTTINGHAM[6] has shown that he can displace more than 90 per cent of the $MgSO_4$ of Knop's solution with $Mg(NO_3)_2$ without interfering with the growth of red clover, a rather heavy sulphur requiring plant. It is evident that some of the nutrients in the commonly used nutrient solutions are far beyond the minimum concentration necessary to give the plant its optimum supply, and that the so-called optimum concentration of the solution is determined by other factors than optimum supply. The conditions of and the mechanism for absorption from the soil (root hairs with their acid pectic layer in contact with soil particles bearing certain nutrients in compounds of low solubility) are quite different. Work with water cultures has established some very fundamental principles in soil fertility (essential nutrient elements, necessity of balanced solutions, etc.). It is a question how much more this method alone is capable of adding to our knowledge of soil fertility. In the present concentrated nutrient solutions with which we are working we may be mainly playing the toxic concentration of one salt against the toxic concentration of another in a way to get the least possible injury.—WM. CROCKER.

Chromosomes in Carex.—Oogenesis and spermatogenesis have been studied by HEILBORN[7] in several species of *Carex*, special attention being given to chromosome numbers, which vary greatly in this genus. The gametophyte numbers in the forms investigated are as follows: *Carex pilulifera* 8, *C. ericetorum* 16, *C. digitata* 24, *C. caryophylla* and *C. flava* 32. JUEL had already reported 52 for *C. acuta*, and STOUT 37 for *C. aquatilis*. It is interesting to note that *C. pilulifera* has the largest chromosomes, and that in species with higher numbers the chromosomes are correspondingly smaller. Attempts to cross the various species have not yet proved successful, but the work is still in progress.—C. J. CHAMBERLAIN.

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[5] TOOLE E. H., and TOTTINGHAM, W. E., The influence of certain added solids upon the composition and efficiency of Knop's nutrient solution. Amer. Jour. Bot. **5**:452–461. 1918.

[6] TOTTINGHAM, W. E., Sulfur requirement of red clover plant. Jour. Biol. Chem. **36**:429–438. 1918.

[7] HEILBORN, OTTO, Zur Embryologie und Zytologie einiger *Carex*-Arten. Svensk Botanisk Tidskrift **12**:212–220. *figs. 1–14*. 1918.

PLANT GENETICS

By JOHN M. COULTER
Head of the Department of Botany in the University of Chicago
and MERLE M. COULTER
Instructor in Plant Genetics in the University of Chicago

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THE BOTANICAL GAZETTE

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JUNE 1919

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STRUCTURE, DEVELOPMENT, AND DISTRIBUTION OF SO-CALLED RIMS OR BARS OF SANIO

IRVING W. BAILEY

(WITH PLATES XIII–XV)

Introduction

In recent years a number of botanists and paleobotanists have given considerable attention to the study of the distribution of certain bandlike thickenings of the middle lamella, so-called rims or bars of Sanio, in the gymnosperms, and their significance in discussion concerning the relative antiquity of the Abieteae and Araucarieae.[1] Before considering the distribution of these bandlike thickenings of the middle lamella, it is desirable to outline the conclusions of various investigators concerning their structure and development. The work of SANIO is particularly significant in this connection, as it is also in a discussion of the controversy that has arisen in regard to the true meaning of the term "bars of Sanio."

Historical

STRUCTURE AND DEVELOPMENT OF SANIO'S QUERLEISTEN.—It was stated by SANIO (13), in his comprehensive paper upon the anatomy of *Pinus silvestris* Linn., that in the cambium of young stems the radial and tangential partitions separating adjacent protoplasts are of equal or nearly equal thickness, but in that of

[1] The terminology of ENGLER and GILG (2) is used in this paper.

old stems the radial walls are considerably thicker. He showed that each of these thick radial partitions is distinctly stratified, and consists of a central cellulose septum, *Zwischensubstanz*, overlaid by cellulose layers belonging to the protoplasts on either side of the wall. Furthermore, he believed that at an early stage in the development of tracheids thin spots appear in the radial primary walls; and that, as the tracheids increase in size, these areas become larger, the *Zwischensubstanz* is gradually absorbed, and the outer layers of the wall are fused together and stretched to form a thin, more or less homogeneous membrane. Inasmuch as bordered pits are subsequently laid down over portions of these attenuated areas, SANIO considered them to be *Primordialtupfeln*. He described them as follows (p. 74):

> Während diese Verdünnungen in der Membran seitlich, d. h., in horizontaler Richtung allmählich in den stärken verdickten Theil übergehen grenzen sie sich Oben und Unten scharf ab, und erscheinen hier sogar zuletzt mit doppelten Umrissen. Häufig liegen diese Verdünnungen so nahe an einander, dass die sie trennenden verdickten Stellen als Querleisten erscheinen. Untersucht man diese Bildung in Tangentialschnitte, so erscheinen diese Querleisten als knotenförmige Verdickungen der Membran zweier Nacharzellen, während die Verdünnungen als zarte Scheidwände sich ausweisen.

It is evident from SANIO's figures and descriptions that he considered the primary pit areas, *Primordialtupfeln*, to be separated by others in which the *Zwischensubstanz* is not entirely absorbed, and in which the outer cellulose layers are less attenuated. It should be emphasized, in this connection, that SANIO used the word *Querleisten*, cross-pieces or cleats, in referring to transverse thicker strips of the middle lamella between closely approximated primary pit areas, and the word *Umrissen*, contours, in referring to the upper and lower outlines of these areas. It is difficult to determine with certainty whether SANIO understood the real significance of the *doppelten Umrissen*, which partially surround the more isolated primary pit areas in certain of his drawings. That he probably considered them to be contours, outlining the top and bottom of a sloping surface or escarpment, is indicated by his illustration (pl. 10, fig. 2) of a tangential section of a young tracheid of *Pinus silvestris*. I have found no conclusive evidence in his text or figures

to indicate that he considered these parallel curved lines as outlines of a heavily embossed or thickened rim.

STRASBURGER (15) showed very clearly that in *Pinus* and *Larix* SANIO'S *doppelten Umrissen* may be the outlines of embossed portions of the middle lamella. In other words, he made it evident that when the primary pit areas are close together they are separated by a single transverse thickening, but that when they are not closely approximated there may be two curved thickened strips, separated by a less heavily embossed area, between them (fig. 5).

Miss GERRY (3), in discussing the distribution of bandlike thickenings of the middle lamella in the Gymnospermae, referred to them as follows (p. 119):

> The "bars" or "folds" of cellulose which when stained with haematoxylin are especially obvious as horizontal or more or less semicircular markings in the tracheide walls of a radial section from such a conifer as *Pinus silvestris* L. were described by SANIO in 1872. . . . . These structures were named "Bars of Sanio" from him. . . . .

GROOM and RUSHTON (5), who have studied the structure and chemical composition of the bandlike thickenings of the middle lamella in Indian species of *Pinus*, state:

> According to SANIO'S work it is these unoccupied margins of the primary areas that coincide in position with the above-mentioned bands that are seen in radial section. Consequently the name "Sanio's rims" may be given to the structures causing the bandlike appearance. . . . . When the primary pit areas are in contact, the two contiguous Sanio's rims are naturally "fused" and form a band that is transverse and single, except possibly at the two lateral edges where the natural curvature of each original boundary of the area causes the band to fork. . . . . In *radial* sections with iodine and sulphuric acid the "rims" stain yellow; with ordinary haematoxylin they remain unstained; leaving sections in cupra-ammonium to dissolve out any cellulose, their staining properties are not changed materially. They are not composed of cellulose. . . . . When young the actual marginal portion of the primary pit area does not thicken by deposits of lignified wall as soon as it does elsewhere (except on the pit-closing membrane), but thickens by successive deposits of pectic substance until a stage is reached when lignified wall-substance is deposited even over the now thickened rims of the primary pit area. Sanio's rims represent a system of rodlike or bandlike pectic thickenings of the middle lamella running transversely in the radial walls and linked here and there by slightly

curved longitudinal bandlike similar thickenings (representing the lateral margins of the primary pit areas). . . . . C. MÜLLER (1890) was the author of the name "Sanio's Bars" and, as he explicitly stated, he coined the term to designate these structures,[2] as first discovered by SANIO in *Pinus silvestris* (1873–74).

JEFFREY (7), however, still maintains that the bandlike thickenings of the middle lamella in the Ginkgoales, Abieteae, Taxoideae, Cupresseae, and Taxaceae are typical "bars of Sanio." SIFTON (14) considers that the rims of neighboring primary pit areas unite to form bars and uses the latter term in referring to bandlike thickenings of the middle lamella that occur in *Araucaria* and *Cycas*.

BARS OF SANIO VS. TRABECULAE.—The fact that the term bars of Sanio is used by certain investigators in referring to trabeculae and by others in describing entirely different structures is unfortunate and likely to lead to considerable confusion. In a paper, published in 1863, SANIO (12, p. 17) described trabeculae as: "Querbalkens quer durch den Zellenraum von einer Wandung zur andern verlaufen." In 1890, MÜLLER (9) referred to these structures as *Sanio'sche Balken;* and later PENHALLOW (10) called them Sanio's bands. It is evident that SANIO and MÜLLER used the word *Balken* (beams) to designate rodlike structures that are attached at their ends and cross the lumens of cells. In view of this fact, and that SANIO used the word *Querleisten* (cross-pieces or cleats) in referring to bandlike thickenings of the middle lamella, the terms "bars of Sanio" and *Sanio'sche Balken* are not necessarily synonymous. As has been pointed out by GROOM and RUSHTON, however, JEFFREY and his students were undoubtedly mistaken in supposing that the bandlike thickenings of the middle lamella had been named after SANIO. This conclusion is strengthened by the fact that the phrase "diese scheibenförmige Verdickung der Scheidewand ist bisher übersehen" (used by SANIO [13, p. 78] in referring to the torus) was interpreted as indicating that SANIO considered himself the discoverer of the bandlike thickenings of the middle lamella; whereas, as a matter of fact, he expressly stated (13, p. 74) that "Deartige Bildungen hat bereits Unger gesehen, aber nicht zu deuten gewusst."

Although the usè of the term "bars of Sanio" was undoubtedly unfortunate, JEFFREY and his students do not appear ever to have

[2] Trabeculae.

actually confused SANIO's *Querleisten* with trabeculae. Furthermore, it is to be emphasized that GROOM and RUSHTON consider the rodlike thickenings, between closely approximated primary pit areas, as fusions of two thickened rims. The word rims, therefore, is not an entirely satisfactory substitute for the word bars in referring to SANIO's *Querleisten*.

DISTRIBUTION AND SUPPOSED PHYLOGENETIC SIGNIFICANCE OF SANIO'S QUERLEISTEN IN CONIFERAE.—There are considerable differences of opinion among various investigators concerning the distribution and phylogenetic significance of these bandlike thickenings of the middle lamella. JEFFREY (7) and his students (3, 6) maintain that they are conspicuously developed in the older wood of *Ginkgo* and all of the Coniferae except the Araucarieae. GOTHAN (4) assumes that they are absent in the Araucarieae because the pits are so closely packed together that there is no room for such structure. THOMSON (16), on the other hand, considers that they are present in rudimentary form in the Araucarieae, and are closely applied to the margins of the bordered pits.

Whatever view is taken in regard to the relative antiquity of the Abieteae and Araucarieae, it must be admitted that there is a very striking difference between the older wood of the Araucarieae and that of the Abieteae, Taxodieae, Cupresseae, Taxaceae, and *Ginkgo*. "Alternate" pitting (fig. 13) is stereotyped in the Araucarieae; whereas "opposite" pitting and *Querleisten* (pl. XV) are firmly fixed in the Abieteae, Taxodieae, Cupresseae, and Taxaceae. There appear to be no true transitional series between these two types of secondary xylems that may be considered to indicate conclusively that the latter type of pitting is a modification of the former.

In so far as tracheary pitting is concerned, the principal arguments in favor of deriving the Abieteae and Taxaceae from the Cordaitales or Araucarieae are based upon the anatomy of the young wood of seedlings, cone axes, and the first annual rings of stems and roots, so-called conservative regions. Thus JEFFREY (7) maintains that the presence of alternate pitting and the absence of bandlike cellulose thickenings of the middle lamella in the young wood of reproductive axes, leaf strands, and the first annual rings of *Ginkgo*

and *Pinus*, are evidences of the Cordaitean ancestry of these genera. He considers the opposite pitting and thickenings of the middle lamella which occur in the cone axes of Araucarians to indicate that the Araucarieae are descended from forms resembling *Ginkgo* and *Pinus*. Furthermore, although admitting that "bars of Sanio" are absent or "evanescent" in the young wood of seedlings and the first annual rings of the stems and roots of Araucarieae, he interprets the absence of approximation and consequent flattening of the bordered pits in such tissue as evidence for deriving the Araucarians from pinelike ancestors.

The accuracy of these conclusions has been questioned by THOMSON (16) and SIFTON (14), who have figured and described bandlike thickenings of the middle lamella in the tracheids of the petiole of *Cycas* and the cone axes, seedlings, and first formed secondary xylem of the stems and roots of *Pinus* and other Abieteae. THOMSON (16) interprets the rimlike thickenings and alternate pitting that occur at times in the cone axes and first annual rings of stems and roots of *Pinus* as indicating that the Abieteae are descended from the Araucarieae.

It is to be emphasized, in this connection, that in dealing with other structural characters JEFFREY interprets the anatomy of selected conservative regions or organs of the Abieteae and Araucarieae as indicating that the latter are descended from the former; whereas THOMSON, by applying the same laws to similar material, proves the reverse to be true.

Such discrepancies as these suggest that there may be a considerable element of danger in placing too much emphasis upon "laws" of recapitulation, reversion, and retention in arguments concerning the phylogeny of plants. Even the most ardent advocates of these doctrines admit, in certain cases at least, that cenogenetic characters do occur in seedlings, roots, traumatic tissue, cone axes, etc. So long as this is acknowledged to be so, it must be extremely difficult, in the absence of reliable collateral evidence, to determine with certainty whether a given structure in a given region is palingenetic or cenogenetic. In other words, even if it should be proven, by means of careful statistical and experimental investigations, that certain organs or regions of plants are inherently

somewhat more conservative or slower to change than others, considerable difficulty must inevitably be encountered in formulating such facts as these into laws for use as "short cuts" in the study of phylogeny.

That this is likely to be the case in dealing with the Ginkgoales and Coniferae is indicated by a number of facts in the comparative anatomy and ecology of the Pteridophyta and Gymnospermae. In the evolution of these groups the primary, as well as the secondary, tissues appear to have been considerably modified. For example, the more primitive vascular plants were characterized by having relatively wide zones of primary xylem; whereas the Coniferae have usually only a relatively limited amount of this tissue, which is correspondingly specialized in structure.

Structure and distribution of bandlike thickenings of middle lamella in Pteridophyta, Gymnospermae, and Angiospermae

In view of the fact that much emphasis has been placed upon bars of Sanio in the identification of fossil woods of the Mesozoic, and that these structures have been used as the basis for important but conflicting generalizations in regard to the phylogeny of the Coniferae and the relative conservatism of different organs or regions of plants, the structure and distribution of bandlike thickenings of the middle lamella in the Pteridophyta, Gymnospermae, and Angiospermae deserve more careful consideration than they have received heretofore.

As is well known, the metaxylem of most Filicales is composed largely of scalariform tracheids. The bordered pits in these tracheary elements are much elongated horizontally, at right angles to the long axis of the tracheids, and are closely approximated in vertical series (fig. 2). The elongated bordering areas of the secondary walls are exactly superimposed over attenuated areas of the middle lamella; and the outlines of these areas are more or less effectively concealed by the margins of the bordering areas. The primary pit areas are separated by narrow, bandlike, thicker portions of the middle lamella, which, in carefully stained[3] longitudinal sections of the xylem, appear as fine dark lines between the

[3] Haidenhain's iron-haematoxylin and safranin.

bordered pits. Owing to the approximation of the bordered pits and the thickness of the secondary walls, however, these *Querleisten* are usually more conspicuous when seen in section (fig. 19) than in surface view.

This scalariform type of tracheary pitting becomes at times considerably modified. Thus the elongated bordering areas of the secondary wall may be replaced by two or more shorter elongated or oval bordering areas (fig. 2). Under these circumstances the primary wall frequently retains its typical scalariform pitting after the secondary wall has lost it; that is to say, each horizontal row of smaller bordering areas is laid down over a single elongated primary pit area (fig. 6). In other cases the elongated bordered pits of the secondary walls may become contracted to form smaller bordered areas which cover only a portion of the surface of the elongated primary pits, and the *Querleisten* project beyond the outlines of the bordering areas. This process of reduction in the pitting of the secondary wall may even be carried to a point where the primary pit areas have no superimposed bordered pits; or the primary pit areas become less closely approximated, of oval or circular outlines, and separated by relatively wide biconcave thickenings with forking ends (fig. 7).

Scalariform pitting also grades into types in which there is less unconformity between the primary and secondary walls. The elongated bordered pits become replaced by vertical rows of smaller pits which are staggered so that the pits in one row alternate with those in the next series. These pits are usually superimposed over nearly the whole surface of similar primary pit areas, and the thicker portions of the middle lamella tend to anastomose or form a reticulum, as is shown in fig. 6*a*.

Such transitions between scalariform and derived types of tracheary pitting occur in other groups of vascular plants. In certain of the paleozoic and lower mesozoic plants, which had "open" bundles, the metaxylem and secondary wood were composed of scalariform tracheids; whereas, in others, the scalariform bordered pits were more or less completely replaced by horizontal or diagonal rows of smaller pits, except in the tracheids of the younger wood of the stele. In the latter types, in passing from the

younger to the older metaxylem or secondary wood, there were transitions between typical scalariform and opposite and alternate multiseriate pitting. Such transitional stages between scalariform and multiseriate pitting have been observed in a number of Sphenophyllales, Calamariales, Cycadofilicales, Cordaitales, and Bennettitales. In *Protopitys*, *Cycadeoidea Dartoni* (Coulter and Chamberlain) Wiel. (figs. 14, 20), and other forms whose secondary xylems show indications of zonation, such transitions occur periodically in the older wood of the stem, as they do in the stems and roots of the vesselless angiosperms, *Tetracentron* and *Trochodendron* (figs. 15, 16). In these transitional regions the elongated primary pit areas and *Querleisten* that underlie the scalariform secondary walls tend to persist in tracheids having horizontal rows of bordering areas.

It is such transitional types of tracheary pitting that have been figured by JEFFREY in the cone axes of Araucarians, and by SIFTON in the petioles of *Cycas*. In *Araucaria Bidwillii* Hook., owing to the fact that the middle lamella is often relatively thick and the pits not closely approximated, the *Querleisten* are frequently broad and conspicuous. In transitional tracheids they may break into fragments which cling to the margins of the bordered pits, even after the latter have shifted to the alternate arrangement. Eventually, however, the more or less circular primary pit areas of the older tracheids appear to become surrounded on all sides by equally thickened portions of the middle lamella.

Scalariform and transitional types of bordered pitting occur in the lateral walls of the vessels of many dicotyledons. In fig. 3 is illustrated the typical scalariform bordered pitting that occurs in the radial and tangential walls of the vessels of certain Magnoliineae. This type of pitting is in marked contrast to the multiseriate pitting shown in figs. 4 and 9. As these figures indicate, the bordered pits which form the horizontal rows may be closely packed together and have flattened sides, or they may be more loosely arranged and have oval outlines. Transitional stages, between these typical scalariform and multiseriate types of pitting, are of frequent occurrence in the vessels of certain of the Magnoliineae (figs. 4, 11).

In photomicrographs of carefully stained sections there are thin dark colored lines between the elongated or scalariform

bordered pits. These transverse strips are deeply stained portions of the middle lamella that stand out in sharp contrast to the thin pit membranes. They are, in fact, very narrow, bandlike thickenings (*Querleisten*) which separate the elongated primary pit areas. In the case of vessels with multiseriate pitting, these transverse ridges or *Querleisten* tend to occur between the elongated primary pit areas that underlie the horizontal rows of bordered pits. In other words, a single elongated bordered pit may be laid down over the whole surface of the elongated primary pit area, or one or more smaller bordered pits may be laid down over portions of its surface. Under the latter circumstances, the outlines (*Umrissen*) of the primary pit areas become more conspicuous (figs. 4, 9, 11). In certain cases these *Querleisten* become more or less completely divided into shorter, rodlike thickenings, which lie between the upper and lower margins of contiguous bordered pits of the vertical series.

It was shown by STRASBURGER (15) that bordered pits are not laid down over all the primary pit areas in the tracheids of *Pinus* and *Larix*. In the Magnoliineae and other dicotyledons the lateral primary walls of the vessels frequently have elongated attenuated areas, which have no bordered pits superimposed over them, or only a comparatively limited part of their surface so covered (figs. 1, 8, 12).

In the Magnoliineae, Trochodendrineae, and other groups of dicotyledons there is much evidence to indicate that scalariform pitting is a relatively primitive feature in the structure of vessels. That is to say, those vessel segments which most closely resemble tracheids in general form and structure tend to have scalariform or scalariform and opposite multiseriate pitting; whereas the larger and more specialized conducting passageways are characterized by having alternate multiseriate pitting in their lateral walls (fig. 10).

In the evolution of larger and more specialized vessels the modification of the primary walls does not appear, in many cases, to have kept pace with that of the secondary walls. Thus in primitive types of vessel segments the elongated bordered pits are exactly superimposed over similar elongated primary pit areas, but in more

specialized types the primary wall (the first formed portion of the vessel member) tends to retain its primitive elongated type of primary pit areas after the scalariform bordered pits have become locally constricted (fig. 4), or divided into horizontal rows of smaller pits (fig. 9). Similarly, in those walls where there is a tendency to eliminate the bordered pits, the elongated primary pit areas persist after the bordered pits have partially or completely disappeared (figs. 1, 8, 9).

In the highly specialized vessels of the Anonaceae and Lauraceae (fig. 10) there are numerous circular or oval bordered pits in the lateral walls of the vessels. Usually they appear to be laid down over similar circular primary pit areas. In other words, in the most highly specialized types of vessels, in which the bordered pits are not arranged in horizontal rows, even the elongated primary pit areas are more or less completely obliterated, and replaced by attenuated areas with circular outlines. Vestiges of *Querleisten*, however, are sometimes present near the upper and lower margins of the bordering areas.

In the dicotyledons, with increasing specialization of the vessels, there is a corresponding reduction in the pitting of the remaining tracheary elements. Thus typical tracheids are replaced by fiber tracheids, which are in turn replaced by libriform fibers. The fiber tracheids of certain dicotyledons have elongated or oval primary pit areas that are separated by wide dark colored bands. Furthermore, it is not uncommon to find that many of the attenuated areas of the middle lamella have no superimposed bordered pits (fig. 22).

Unconformity of the type that occurs in the tracheids of various primitive vascular plants, and in the vessels of certain dicotyledons, has been observed in the wood of *Ginkgo*, and certain of the Abieteae and Taxodieae. Fig. 23 illustrates a type of tracheary pitting that is of frequent occurrence in the older secondary wood of vigorous mature specimens of *Ginkgo* and *Taxodium*. It is most characteristically developed in large thin-walled tracheids of the so-called spring wood. The numerous, uniformly narrow, elongated primary pit areas and thin, straight, narrow *Querleisten* are typically scalariform in structure. Each primary pit area has superimposed

over it 2–4 bordered pits. The latter are somewhat elongated in many cases, and frequently are so closely approximated as to be flattened by mutual contact and to cover nearly the whole of the primary pit areas. This type of tracheary pitting grades into a second type in which the surfaces of the elongated primary pit areas are only partially covered by circular bordered pits (figs. 24, 25). The latter type, in turn, grades into a third type in which the primary pit areas are not typically scalariform in structure. Certain of the attenuated areas appear to increase in size at the expense of intervening areas, which are either eliminated entirely or persist as constricted areas that are not overlaid by bordered pits (figs. 26, 29, 30). By this process of specialization certain of the primary pit areas become oval or biconvex and less closely approximated. Certain of the *Querleisten* tend to widen and to become biconcave with forking ends; whereas others are crowded together and appear to fuse to form similar biconcave thickenings (figs. 26, 29, 31). This type of pitting grades into others in which the attenuated areas that are overlaid by bordered pits become more circular and more widely separated, the intervening primary pit areas and *Querleisten* (except the curved bands that commonly persist above and below the bordered pits) becoming vestigial or obliterated (figs. 27, 28). In certain cases the portions of the middle lamella between widely separated primary pit areas may be uniformly thickened so that the pits appear to be separated by a single, wide dark colored band. In other cases, for example, in the thick-walled fiber-like cells of the so-called summer wood, and in the small tracheids which occur in seedlings and the first annual rings of stems and roots, the bordered pits frequently tend to be superimposed over nearly the whole surface of the circular primary pit areas, and the curved *Querleisten* cling to the upper and lower margins of the bordering areas, or are completely obliterated.

Such transitions in tracheary pitting have been observed in *Larix*, *Pinus*, *Abies*, *Sequoia*, and other genera of the Abieteae and Taxodieae, as well as in *Taxodium* and *Ginkgo*. The more elongated primary pit areas and the narrower, straighter *Querleisten* tend to occur in the larger, thinner-walled, heavily pitted tracheids; and are therefore most characteristically developed in the first formed

portions of the growth rings of the older secondary xylem. The particular types of unconformity and bandlike thickenings of the middle lamella which occur in a given species vary considerably in plants grown under different environmental influences and in different organs or regions of a single plant. This is as true of the first formed as the older secondary xylem.

Discussion

It is evident that bandlike thickenings[4] of the middle lamella, separating more or less elongated primary pit areas, are not confined to the tracheids of certain Coniferae, but are widely distributed among the Pteridophyta, Gymnospermae, and Angiospermae.[5] Any interpretation of the so-called rims or bars of Sanio in the Coniferae, therefore, should be in general harmony with the structure and distribution of these bandlike thickenings in other groups of vascular plants.

Since bandlike thickenings of the middle lamella and transitions between scalariform and "alternate" and "opposite" multiseriate pitting are of common occurrence in the younger xylem of many paleozoic and mesozoic (as well as less primitive) plants, the occurrence of opposite (as well as alternate) pitting and more or less rudimentary *Querleisten* in the transitional tracheids of the cone axes of Araucarians does not indicate conclusively that the Araucarieae are descended from the Abieteae. Similarly, the more or less sporadic occurrence of alternate pitting, as well as opposite pitting and "bars of Sanio," does not indicate necessarily that the secondary xylem of the Ginkgoales, Abieteae, Taxodieae, Cupressеae, and Taxaceae is a modification of that which occurs in the Araucarieae or Cordaitales.

Furthermore, there are a number of facts in the comparative anatomy and ecology of the Pteridophyta, Gymnospermae, and Angiospermae which suggest that unconformity between the pits in the primary and secondary walls of tracheids and vessels may be

[4] Which stain dark blue in sections treated with Haidenhain's iron-haematoxylin.

[5] The bandlike thickenings are usually inconspicuous in surface views of the facets of tracheary elements owing to the fact that they are concealed by the thick, superimposed secondary walls.

a phenomenon that is concomitant of processes of modification or reduction in tracheary pitting. In the metaxylem of ferns and in the secondary xylem of a number of primitive vascular plants, the primary wall frequently tends to retain its scalariform structure after the scalariform bordered pits in the secondary wall have been replaced by horizontal rows of smaller pits. It may even retain its elongated pit areas and bandlike thickenings after the bordering areas of the secondary wall have been considerably contracted, or have disappeared entirely from certain portions of a facet. On the other hand, when scalariform pitting is replaced by alternate multiseriate pitting, the bandlike thickenings of the middle lamella tend to anastomose and form a reticulum.

Similar phenomena occur in the metaxylem of many of the higher vascular plants, in the tracheids of the secondary xylem of the vesselless dicotyledons *Tetracentron*, *Trochodendron*, and *Drimys*, and in the lateral walls of the vessels of many of the angiosperms.

This general tendency for the persistence of scalariform pitting in the middle lamella, after it has disappeared more or less completely from the secondary wall, raises an interesting question in regard to the probable significance of the scalariform pitting which occurs so commonly in the middle lamellae of certain of the Abieteae, Taxodieae, Taxaceae, Cupresseae, and *Ginkgo*, but appears to be entirely absent in the later formed secondary tracheids of the Araucarieae.

It is important to note in this connection that the more primitive vascular plants, which possessed relatively wide zones of primary xylem, were characterized by having numerous closely approximated pits in the radial facets of their relatively large tracheids. In the evolution of the Ginkgoales and Coniferae there appears to have been a more or less pronounced reduction in the amount of primary xylem, in the size of the first formed secondary tracheids of the stele, and in the number of bordered pits in the walls of the tracheary elements.

The large, thin-walled, heavily pitted tracheids which occur in the spring wood of the older secondary xylem of mesophytic Coniferae, resemble the primitive types of tracheids more closely than do the thick-walled, highly specialized elements of the summer wood,

or the relatively small tracheids of the first formed portion of the secondary xylem. It is in these larger thin-walled tracheids that the most typical scalariform primary pit areas tend to occur. Occasionally, where the tracheary pitting is very strongly developed, scalariform bordering areas of the secondary wall are superimposed over portions of these elongated primary pit areas (fig. 23). This is likely to occur in *Taxodium* and *Ginkgo* and roots of certain of the Abieteae. As has previously been shown, the scalariform structure of the middle lamella and narrow straight *Querleisten* become gradually modified with increasing reduction in the number of bordered pits. Furthermore, it has been shown that a similar widening of the bandlike thickenings of the middle lamella may occur in certain of the Pteridophyta, as well as the Angiospermae, when the bordered pits tend to become more or less isolated.

The occurrence of these interesting structures in the Abieteae, Taxodieae, Cupresseae, Taxaceae, and *Ginkgo*, and their absence in the secondary wood of Araucarieae, are difficult to explain upon the assumption that the former groups are descended from ancestors having "alternate multiseriate" pitting. On the other hand, from analogy with similar phenomena in other groups of vascular plants, their occurrence is easily accounted for if the microphyllous and relatively xerophytic Coniferae are descended from forms having scalariform tracheary pitting.

Primary pit areas of cambium and their relation to the pitting of xylem and phloem

The important observations of De Bary, Janczewski, Russow, Strasburger, Kienitz-Gerloff, Krüger, and others upon the occurrence of bandlike thickenings of the middle lamella in the cells of the cambium, and their relation to similar structures in the elements of the xylem and phloem, have been overlooked entirely in discussions concerning the phylogenetic significance of the so-called rims or bars of Sanio. Krüger (8) found that, in the cambia of all plants (stems and roots of gymnosperms, dicotyledons, monocotyledons, including trees, shrubs, herbs, and succulents) investigated by him, there were *leistenformige Verdickungen* in the radial partitions (figs. 17, 18). These ridgelike thickenings separated roundish

thin spots or pit areas and were more conspicuous in the winter than in the spring and summer, a fact previously noted by DE BARY (1). Furthermore, KRÜGER traced these structures to the "procambial strands" and through the developing daughter cells of the cambium to the highly differentiated cells of the xylem and phloem. They appeared to be least modified in the walls of phloem parenchyma. In the development of tracheids, vessels, and sieve tubes, owing to increase in the size of the individual cells, they tended to become more or less modified. KRÜGER concluded, however, that the thin spots became enlarged and modified to form sieve plates (fig. 21), and to form the primary pit areas of tracheary elements. He noted that during this process the ridgelike thickenings tended to become more or less modified in shape. RUSSOW (11) and STRASBURGER (15) emphasized the fact that in the Abieteae many of the pit areas become more or less obliterated in the development of tracheids and sieve tubes; that is to say, many of the primary pit areas become vestigial, since they have no superimposed bordered pits or do not become modified to form sieve plates.

It is evident, therefore, that not only are bandlike thickenings of the middle lamella of frequent occurrence in the tracheary elements of Pteridophyta, Gymnospermae, and Angiospermae, but also in the cells of the cambium and phloem. In discussing the phylogenetic significance of the bandlike thickenings of the middle lamella in tracheids of Coniferae, therefore, it is essential, not only to compare these structures with similar structures which occur in other types of cells and other groups of plants, but also to contrast the various stages in their development. This must inevitably be the case, since there is a considerable element of danger in basing generalizations concerning relationships upon comparisons between the structure of end products. Of course similar structures may be attained through entirely different developmental stages. Unfortunately, comparatively little is known in regard to the detailed structure of the cambium and the various stages in the development of tracheary pitting in different groups of vascular plants. It is to be hoped that this gap will be filled in the near future, since information of this character promises to throw con-

siderable light upon a number of interesting problems, especially upon the structure and true significance of the so-called rims or bars of Sanio.

Summary

1. Bandlike thickenings of the middle lamella and scalariform primary pit areas are characteristic of tracheids which have scalariform bordered pits. They are widely distributed among the Pteridophyta, Gymnospermae, and Angiospermae.

2. The middle lamella frequently retains its typical scalariform structure after the secondary wall has lost it.

3. In the Gymnospermae, as well as in the Pteridophyta and Angiospermae, there appear to be transitions between primary membranes of this type and others in which the scalariform structure is profoundly modified.

4. The comparative anatomy and ecology of the Pteridophyta, Gymnospermae, and Angiospermae afford considerable evidence which suggests that the types of unconformity and peculiar bandlike thickenings of the middle lamella (so-called bars or rims of Sanio) which occur in certain Pteridophyta and Angiospermae, as well as in many Gymnospermae, are concomitants of processes of modification or reduction in tracheary pitting.

5. The structure of the walls of the cambium and the development of the pitting in the elements of the xylem and phloem in Pteridophyta, Gymnospermae, and Angiospermae deserve more careful consideration in discussions concerning the phylogenetic significance of the so-called rims or bars of Sanio, than they have received heretofore.

Bussey Institution
Jamaica Plain, Mass.

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EXPLANATION OF PLATES XIII–XV

PLATE XIII

FIG. 1.—*Magnolia acuminata* Linn.: pitting in facet of small vessel segment, showing scalariform primary pit areas and isolated circular bordered pits; ×300.

FIG. 2.—*Todea hymenophylloides* A. Rich.: pitting in lateral facet of tracheid, showing typical scalariform and transitional types of pitting; ×300.

FIG. 3.—*Magnolia acuminata:* scalariform pitting in lateral facet of vessel segment; ×400.

FIG. 4.—*Magnolia acuminata:* pitting in lateral facet of vessel segment, showing scalariform and transitional types of pitting; ×450.

FIG. 5.—*Pinus Strobus* Linn.: tangential longitudinal section, showing *knotenformige* thickenings of middle lamella; ×500.

FIG. 6.—*Todea hymenophylloides:* pitting in lateral facet of tracheid, showing pairs of elongated bordering areas superimposed over single scalariform

primary pit areas; portions of bandlike thickenings of middle lamella visible between rows of bordered pits; ×300.

Fig. 6*a*.—*Todea hymenophylloides:* showing alternating arrangement of bordered pits and anastomosing of bandlike thickenings of middle lamella; ×300.

Fig. 7.—*Acrostichum sorbifolium* Linn.: lateral facet of tracheid, showing small, circular, bordered pits and biconcave thickening of middle lamella, latter somewhat indistinct owing to thickness of superimposed secondary wall; ×800.

Fig. 8.—*Cercidiphyllum japonicum* Sieb. and Zucc.: lateral facet of vessel segment, showing persistence of scalariform primary pit-areas after more or less complete disappearance of bordered pits; ×300.

Fig. 9.—*Magnolia macrophylla* Michx.: lateral facet of vessel segment, showing unconformity between primary and secondary pitting.

Fig. 10.—*Asimina triloba* (Linn) Dun.: lateral facet of vessel segment, showing alternate multiseriate pitting; ×300.

Fig. 11.—*Cercidiphyllum japonicum:* lateral facet of vessel segment, showing transitional types of bordered pitting and unconformity between primary and secondary walls; ×400.

Fig. 12.—*Cercidiphyllum japonicum:* lateral facet of vessel segment, showing unconformity between pitting of primary and secondary walls; ×400.

PLATE XIV

Fig. 13.—*Agathis australis* Steud.: pitting in radial facet of tracheid, showing "alternating" type of arrangement; ×350.

Fig. 14.—*Cycadeoidea Dartoni* (Coult. and Chamb.) Wiel.: radial longitudinal section of secondary xylem, showing transitional types of pitting; ×100.

Fig. 15.—*Trochodendron aralioides* Sieb. and Zucc.: radial facets of 2 tracheids, showing transitions between scalariform and opposite bordered pitting; *Querleisten* appear as narrow dark lines separating scalariform pits or pairs of smaller bordered pits; ×350.

Fig. 16.—*Trochodendron aralioides:* radial longitudinal section of secondary xylem, showing transitional types of pitting and (right) persistence of scalariform primary pit areas with reduction in number of bordered pits; ×200.

Fig. 17.—*Ulmus americana* Linn.: tangential longitudinal section of cambium (winter condition), showing *knotenformige* thickenings of middle lamella; ×230.

Fig. 18.—*Ulmus americana:* radial longitudinal section of cambium and young phloem cells, showing sculpture of middle lamella; ×200.

Fig. 19.—*Pteris aquilina* Linn.: adjacent walls of 2 tracheids in sectional view, showing *knotenformige* thickenings ("bars of Sanio") of middle lamella; ×300.

FIG. 20.—*Cycadeoidea Dartoni:* radial longitudinal section of secondary xylem, showing transitions between scalariform and opposite and alternate pitting; ×100.

FIG. 21.—*Juglans nigra* Linn.: radial longitudinal section through cambium (left) and young phloem (right), showing relation between sieve plates and primary pit areas; ×180.

FIG. 22.—*Kayea paniculata* Merrill: radial longitudinal section of secondary xylem, showing primary pit areas and "bars of Sanio"; ×200.

PLATE XV

FIG. 23.—*Taxodium distichum* (Linn.) Richard: radial facet of tracheid, showing scalariform structure of primary wall and crowded bordered pits; ×400.

FIG. 24.—*Larix occidentalis* Nutt.: radial facets of 3 tracheids, showing scalariform structure of primary wall; ×350.

FIG. 25.—*Taxodium distichum:* radial facet of tracheid, showing scalariform structure of primary wall and less crowded bordered pits than fig. 23; ×400.

FIG. 26.—*Larix occidentalis:* radial facets of 2 tracheids, showing numerous primary pit areas having no superimposed bordered pits; ×220.

FIG. 27.—*Pinus Strobus:* radial facets of 3 tracheids, showing modified types of primary wall structure; ×220.

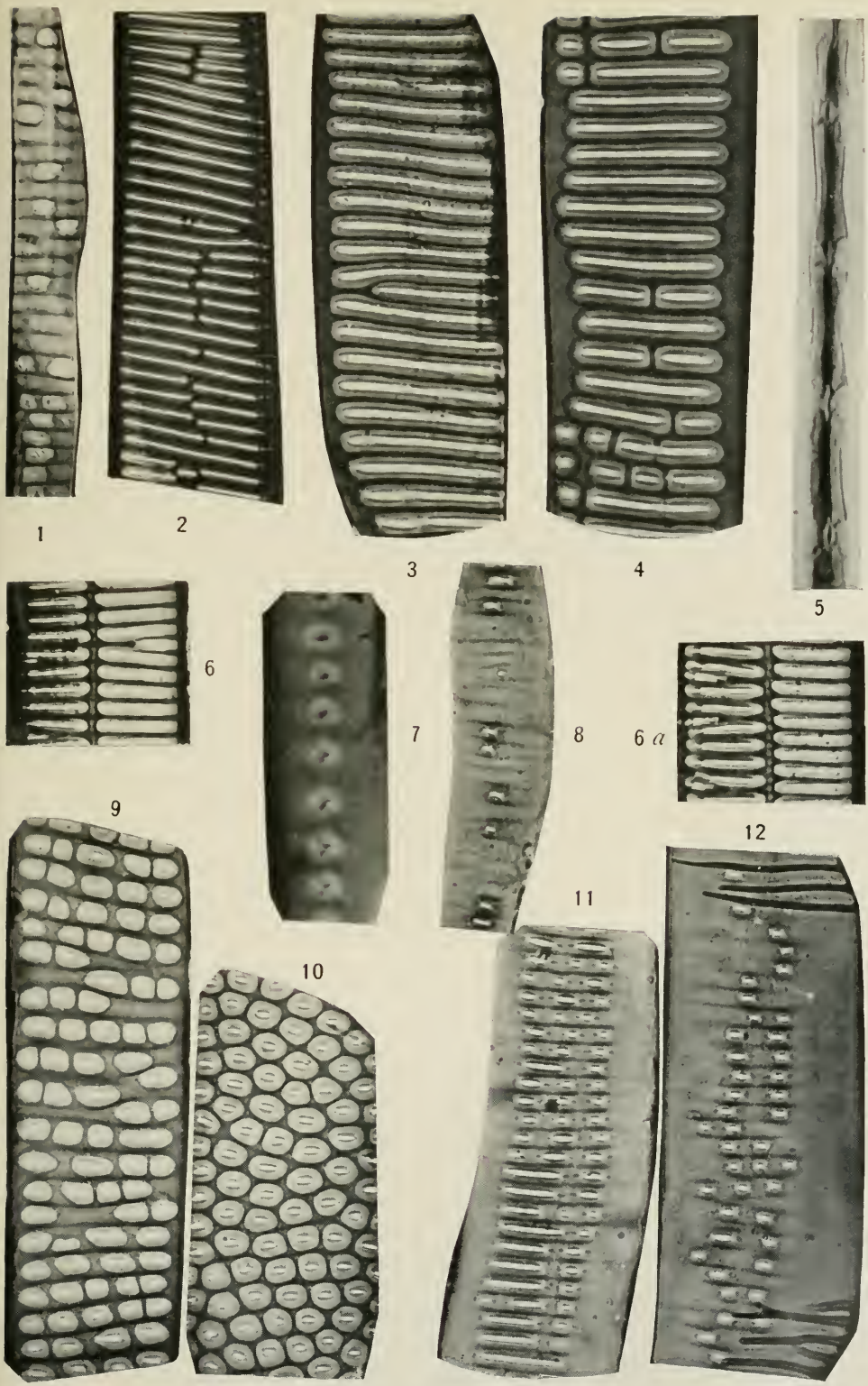
FIG. 28.—*Pinus Strobus:* radial facets of 3 tracheids, showing reduction in bordered pitting and persistence of primary pit areas; ×220.

FIG. 29.—*Larix occidentalis:* radial facet of tracheid, showing curving and fusing (or widening) of bandlike thickenings of middle lamella; ×240.

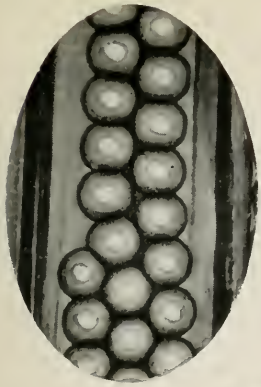
FIG. 30.—*Pinus Strobus:* radial facets of several tracheids, showing scalariform structure of primary wall; ×220.

FIG. 31.—*Taxodium distichum;* radial facet of tracheid, showing curving of bandlike thickenings of middle lamella with reduction in number of bordered pits; ×400.

FIGS. 14, 17, 18, 20, and 21 were made from sections loaned to the writer by Drs. G. R. WIELAND and L. H. MACDANIELS.



BAILEY on BARS OF SANIO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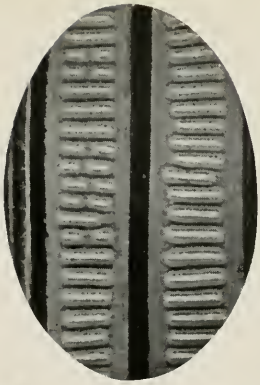
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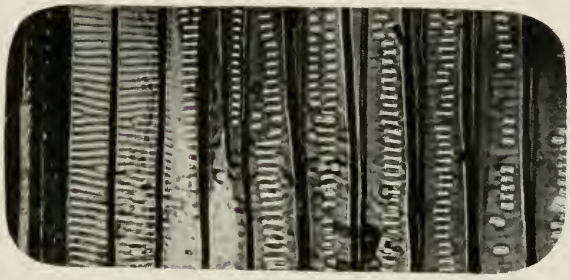
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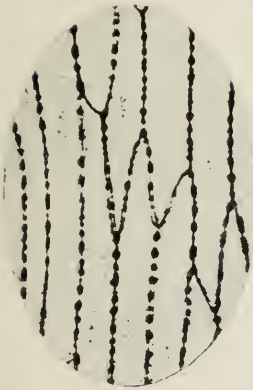
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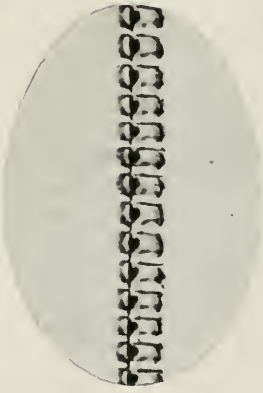
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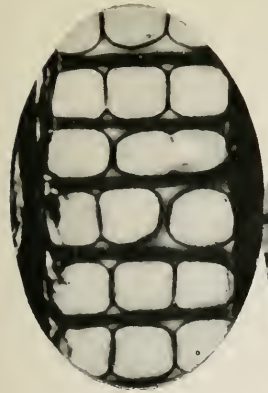

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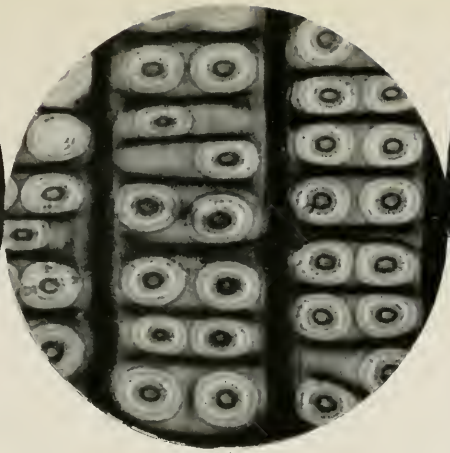

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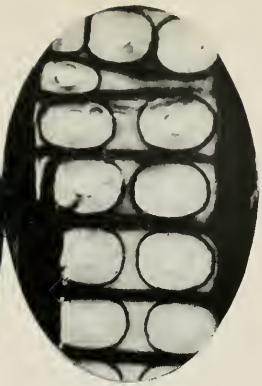

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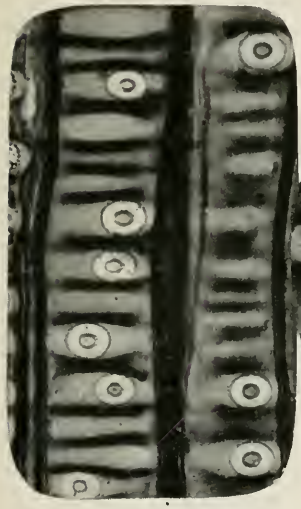

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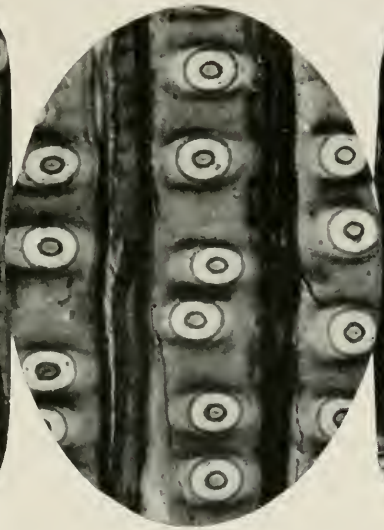

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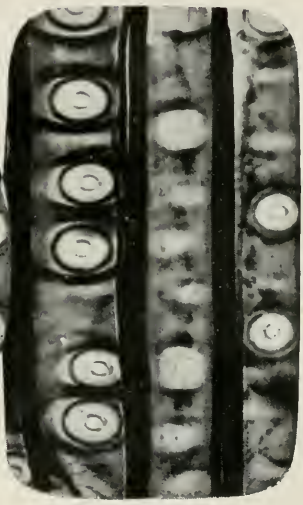

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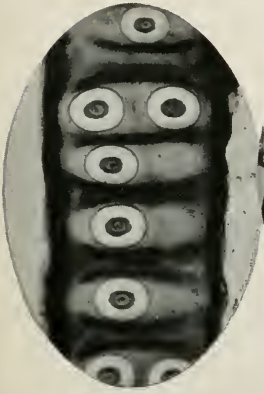

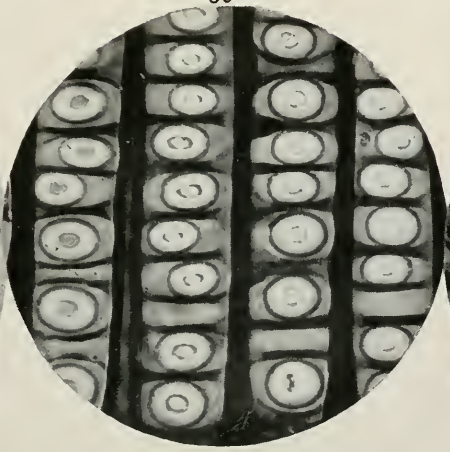

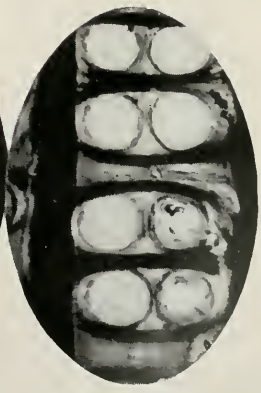

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BAILEY on BARS OF SANIO

APOSPORY IN PTERIS SULCATA L.

W. N. Steil

(WITH PLATES XVI, XVII, AND FOUR FIGURES)

Historical

Although apospory was discovered in the mosses by Pringsheim (18) and Stahl (19) a short time after apogamy had been found in *Pteris cretica albo-lineata* by Farlow (11), apospory remained unknown in the Pteridophytes until Druery (6, 7) reported the phenomenon in *Athyrium Filix-foemina* var. *clarissima* Jones. Prothallia of this fern were observed to form either from the head or the stalk of the sporangium which was arrested in its development. The prothallia of aposporous origin produced antheridia and archegonia. Druery (8) also reported the first case of apical apospory, namely in *Polystichum angulare* var. *pulcherrimum* Wills. The tips of the leaves of this species of fern produced the gametophyte as a direct vegetative outgrowth.

Bower (2) published a brief summary of his investigations of apospory in *Athyrium Filix-foemina* var. *clarissima* Jones and *Polystichum angulare* var. *pulcherrimum* Wills, the material for the investigation having been placed at his disposal by Druery. Sex organs were also observed to develop on the prothallia of the *Polystichum angulare* variety. The main portion of the paper, however, is not concerned with original studies, but with a discussion of "short cuts" in the life history of the fern.

Apospory was also discovered by Bower (3) in *Trichomanes pyxidiferum* and *T. alatum*. In the former the gametophyte generation was produced from aborted sporangia; in the latter there was soral and apical apospory. Sex organs were formed in *T. pyxidiferum*, but in *T. alatum* archegonia were absent and antheridia were never developed to maturity. Only in *T. alatum* were sporophytes observed to develop from the aposporously produced prothallia, and these sporophytes were of apogamous origin. Bower did not seem to be convinced that apospory and

apogamy in these cases were not induced by cultural conditions. He believes that if apospory might be expected to occur anywhere in the plant kingdom, it is in the Hymenophyllaceae, the gametophyte and sporophyte of which are more nearly alike in some respects than in any other homosporous ferns. BOWER (4) later attempted to induce apospory in more than 46 species and varieties of ferns by placing the fronds with immature sporangia on moist sphagnum. In not a single instance, however, were gametophytic outgrowths obtained. In regard to the difficulty with which apospory is induced, he makes the following statement: "There is a marked disability on the part of ferns to bridge over the limits of the two generations by other means than by the formation of spores; the phenomenon is by no means a promiscuous one occurring readily and often, but a rare process, which seems to appear spontaneously under conditions not yet understood and is not readily induced."

FARLOW (12) found apospory in *Pteris aquilina* L. Prothallial growths were produced from sporangia which had aborted in their development. No advanced stages in the development of the prothallia were observed.

The first case of apospory from the young sporophyte of a fern was discovered by DRUERY (9) in a *Lastrea* variety (probably *Lastrea pseudomas* var. *cristata*). Later DRUERY (10) also induced gametophytes aposporously by placing on moist soil portions of the leaves of *Scolopendrium vulgare* var. *crispum Drummondae*. The prothallia of this species developed both sex organs. Apical apospory was reported by DRUERY in *Athyrium Filix-foemina* var. *clarissima* Bolton. He grew sporophytes from the aposporously produced prothallia of *Lastrea pseudo-mas* var. *cristata*. These were of apogamic origin and of interest since they possessed characters of both sporophyte and gametophyte.

STANSFIELD (20) brought into contact with the soil portions of the fronds of *Athyrium Filix-foemina* var. *uncoglomeratum* and thus induced gametophytes aposporously. When "pinnulets" and "leaflets" of young sporophytes, produced by the prothallia of aposporous origin, were pinned down to the soil, he again obtained readily the gametophyte generation. From the young sporophytes

of 4 forms of *Polystichum angulare*, one of *Lastrea*, and 3 of *Athyrium Filix-foemina*, STANSFIELD in a similar manner induced apospory. He is inclined to believe that apospory may be induced by the same method in many other species of ferns.

GOEBEL (14) discovered apospory in *Asplenium dimorphum*. The ends of a finely divided leaf of a plant of this species produced prothallia, typically heart-shaped, and bearing both archegonia and antheridia. The nuclear history was not investigated, but GOEBEL suggests that reduction theoretically occurs when the prothallia are formed. GOEBEL (15) successfully induced apospory in *Aneimia Dregeana*, *Alsophila van Geertii*, *Ceratopteris thalictroides*, *Gymnogramme chrysophylla*, *Polypodium aureum*, and *Pteris longifolia*. In *Marsilia Drummondii* and two *Adiantum* species the results were negative. The primary leaves of young sporophytes were removed and placed on sterilized loam and peat. From the lamina and petiole of the leaf, thus treated, there were produced gametophytes, sporophytes, or forms intermediate in character, since such outgrowths in some cases bore both antheridia and stomata. The aposporously produced prothallia of *Pteris longifolia* developed antheridia and archegonia. Sporophytes were not, however, observed to develop from the prothallia. Since there was found no great difference between the nuclei of the two generations, GOEBEL concludes that there is no sharp line of demarcation between gametophyte and sporophyte. As a result of a series of experiments, GOEBEL found that young sporophyte tissue possesses greater power of regeneration than old tissue. Contrary to BOWER's view that apospory is induced with difficulty and is rare, GOEBEL is convinced that the phenomenon can be produced readily and is widely distributed in ferns. Considered from the phylogenetic point of view, GOEBEL regards the prothallium of the fern as a rudimentary leaf, bearing sexual organs.

In a preliminary note on the cytology of apospory, Miss DIGBY (5) described her results in inducing apospory in *Lastrea pseudo-mas* var. *cristata*, the aposporal nature of which first had been reported by DRUERY (9). As a result of the study of the nuclear condition of the fern, it was reported that 50 chromosomes were retained throughout the life cycle.

FARMER and DIGBY (13) published some very interesting results of their cytological studies of apospory and apogamy in ferns. Five of the 7 species which they investigated produced gametophytes aposporously. The aposporous nature of the 4 following was observed first by DRUERY: *Athyrium Filix-foemina* var. *clarissima* Jones, *A. Filix-foemina* var. *clarissima* Bolton, *Scolopendrium vulgare* var. *crispum Drummondae*, and *Lastrea pseudomas* var. *cristata apospora*. The fifth, *Athyrium Filix-foemina* var. *uncoglomeratum*, was first observed as an aposporous form by STANSFIELD (20). The origin of the aposporously produced prothallia in the different ferns was studied with special reference to changes in the chromosome number, and it was discovered that either the haploid or the diploid number is retained throughout the life cycle. In *Athyrium Filix-foemina* var. *clarissima* Jones, the embryo formed by the prothallia of aposporous origin is apogamic. Ninety chromosomes, the diploid number, are found in both generations. The embryo of *Lastrea pseudo-mas* var. *cristata apospora* is also of apogamic origin, but the chromosome number, between 60 and 78, is probably the reduced number. The other 3 species were found to be parthenogenetic. In *Athyrium Filix-foemina* var. *clarissima* Bolton, 84 chromosomes were counted, in *A. Filix-foemina* var. *uncoglomeratum* about 100, and in *Scolopendrium vulgare* var. *crispum Drummondae* 70 chromosomes were found in the gametophyte, and between 80 and 100 in the embryo sporophyte. Since 64 chromosomes were present in the sporophyte of this species, FARMER and DIGBY were inclined to believe that the diploid number of chromosomes are present.

WORONIN (12, 24) studied apogamy and apospory in the following species of ferns: *Trichomanes Kraussii*, *Pellaea flavens*, *P. nivea*, *P. tenera*, *Notholaena Eckloniana*, and *N. sinuata*. In *Trichomanes Kraussii* sporangia were not produced, but prothallia were formed in large numbers from portions of leaves which were brought into contact with the soil. According to WORONIN, antheridia were in some instances produced directly from the leaves of the sporophyte. It appears, however, that there is not an omission of all the prothallial portion, but that there is formed in each case a short filament which may not be considered as the stalk of an antheridium.

Apospory was induced in *Pellaea flavens* by growing prothallia in continued darkness. When prothallia were transferred to sand cultures there were produced some aposporous growths, but the tendency to apospory was not pronounced. The aposporously produced prothallia in both species in turn produced apogamous embryos and antheridia. Aposporous prothallia were also induced as a result of regeneration experiments. When a portion of the sinus of the prothallium with a young embryo was removed and maintained in culture, aposporous prothallia were occasionally formed. When primary leaves were cut off and similarly placed under cultural conditions, prothallia were produced which developed antheridia but no apogamous embryos.

Materials and methods

A large number of cultures of *Pteris sulcata* L. were made during the past 3 years by sowing the spores on sterilized sphagnum saturated with a one-tenth of 1 per cent Knop's solution or Beyerinck's solution as modified by Moore (17). The spores for the cultural work were obtained from Dr. A. B. Stout, Bronx Botanical Garden, New York, and Dr. E. B. Copeland, Los Baños, Philippine Islands. An abundant supply of spores was also obtained from a plant grown in the university greenhouse.

In one of the cultures made March 1, 1916, the aposporous developments to be described in this paper appeared. Many young apogamously produced sporophytes were found in the culture and on January 1, 1917, some of the young embryos presented a somewhat unusual appearance. As a result of microscopical examination it was discovered that prothalloid portions were present in certain parts of the sporophytes. In June 1917 a number of aposporously developed prothallia were found to be present. When some of the prothallia produced by the germination of the spores were transferred to a new culture, made in a similar manner to that of the original one, more aposporous developments were produced.

The prothallia and embryos from which the drawings and photographs were made were fixed in chrom-acetic acid solution diluted with 5 parts of water, stained with Haidenhain's iron haematoxylin,

and mounted in glycerine jelly. Some of the drawings were made from living material. The sporophyte tissue is represented in the drawings by the darker shading; the gametophyte by the lighter shading.

Observations

The gametophyte of *Pteris sulcata* produced by the germination of a spore is in many respects similar to that of *Pteris cretica* var. *albo-lineata* described by FARLOW (11) and DEBARY (1). When the prothallia of both species were grown under the same cultural conditions, it was observed that those of the former were somewhat larger. Archegonia were never found on any of the large number of prothallia carefully examined with the microscope. Antheridia occur commonly and frequently in great abundance. The antherozoids are apparently normal in every respect.

The embryo of *Pteris sulcata* is always produced apogamously. Such a development of the embryo was first described by the writer (21). SUMINSKI (22) and later MERCKLIN (16) observed tracheids in the prothallia of this species just posterior to the apical notch. DEBARY grew the prothallia of the same species, but observed no sporophytic tissue elements or any other indications of a sporophyte of apogamous origin. Accordingly he reported the fern as non-apogamous. The writer, however, has found the fern to be constantly apogamous under normal cultural conditions. From the sporophytes apogamously produced the gametophytic developments herein described appeared.

In every instance in my culture the prothallial portions or prothallia were produced in connection with the lamina or the petiole of the primary leaf. Usually the terminal portion of the leaf was in part or wholly prothalloid. Occasionally forms were observed which were intermediate in character, the cells partaking of the nature of both sporophyte and gametophyte. Sometimes root, lamina, and petiole of the sporophyte were formed (fig. 13). In some instances the root was absent (figs. 6, 8), and in still other cases only a well developed petiole was present (fig. 8), the lamina of the ordinary leaf being displaced by a prothalloid portion. The vascular system in the primary leaf as a rule was well developed.

Prothallia were found, in one instance, growing from both surfaces of the leaf (fig. 5). These closely resembled the prothallia grown from the spore. The largest one of these was ribbon-like, but the others were mere filaments, each consisting of a single row of cells. On the former an antheridium (*a*) was produced. Rhizoids had been formed from the same prothallium. Two antheridia (*a*, *a'*) had also been developed by a filament produced from the other side of the leaf. From one of these the antherozoids were discharged when the living prothallia, still attached to the lamina of the leaf, were examined under the microscope. The antherozoids were normal in appearance and actively motile. The prothallial growths were surrounded at their points of origin by normal sporophyte cells.

The gametophytic portions, present on the lamina of the primary leaf, may be large, as represented by figs. 6 and 7, in each case of which the terminal portion is distinctly prothalloid. In addition to the large gametophytic portions, a number of smaller regions of the same nature were present on the lamina of the sporophyte represented by fig. 7 (*c*, *d*, *e*, *f*) and a single one by fig. 6, *b*. These were almost wholly surrounded by sporophytic cells. A highly magnified view of *b* is represented by fig. 9. There is, as the figure shows, a sharp line of demarcation between the cells of the two generations. The cells of the gametophytic portion slightly project above the surrounding sporophyte cells. The smaller areas (fig. 7, *e*, *f*) were partially surrounded by cells of a somewhat intermediate character (*n*). The chloroplasts in these cells were less numerous and smaller than those in the neighboring gametophyte cells (fig. 10). Even in the living condition this area was almost colorless as compared with the dark green gametophytic areas, and could be readily distinguished with a hand lens. A cell of the same character as those in the paler region just described was found, in one instance, in the larger gametophytic portion represented by fig. 7. This cell was wholly surrounded by ordinary gametophyte cells (fig. 1). From the lower part of the petiole, represented by fig. 6, a prothallial portion (*p*) had been formed. A leaflike and sporophytic growth (*s*) had also been produced in this case. Three projections

(*m*, *m'*, *m''*), of the nature of secondary prothallia, had also been produced from the terminal gametophytic portion.

The lamina of the leaf in some cases was wholly absent, the petiole being, however, well developed and resembling that of an ordinary leaf (fig. 16). From the ventral surface of each of the two large terminal gametophytic portions (*b*, *c*) numerous antheridia (*a*) had been formed. From the dorsal surface of one of the prothallial portions (*b*) two small secondary prothallia (*m*, *m'*) had begun their development. The vascular system, which was generally well developed in this portion of the embryo sporophyte, extended for some distance into the terminal gametophytic portion. Between the gametophytic and sporophytic portions the cells were of an intermediate character, as shown in fig. 2, which is a highly magnified portion taken at *t*. From the petiole of the same sporophyte a prothallial portion (*p*) had been produced. This outgrowth bears no relation to the vascular system of the petiole. The much modified primary leaf just described was developed in connection with a root and normal secondary leaves.

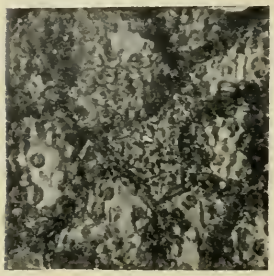

FIG. 1.—Cell of somewhat intermediate character surrounded by ordinary gametophyte cells; ×210.

A structure similar to the preceding one is represented by fig. 8. The petiole appeared to be well developed but both lamina and root were absent. The terminal portion was also distinctly gametophytic in nature and on both surfaces numerous antheridia (*a*) of different sizes and secondary prothallia had been produced.

FIG. 2.—Peculiar cells, intermediate in character, of *Pteris sulcata;* ×162.

An interesting form is represented by fig. 13, since the single large gametophytic portion has been produced in connection with

both petiole and lamina of the leaf. The development of the gametophytic portion was observed for several months and during this period grew rapidly. In the meantime the first secondary leaf had been produced. From both surfaces of the prothallium projections appeared, one of which resembled a young sporophyte (*s*). In this case a root (*r*) had been produced. The two projections (*o*) on the same surface of the prothallium (fig. 15) were similar to those appearing on the other surface, which with a small portion of the prothallium are shown highly magnified in fig. 14. While there is no marked difference between the form of the cells of the outgrowth and the prothallium, those of the latter are much larger. Whether any or all such projections produce embryos was not determined. If the aposporously produced prothallia of *Pteris sulcata* are like those developed from the germination of a spore, and I am inclined to think that they are, such growths may produce prothallia, cylindrical in form, sporophytes normal in every respect, or forms intermediate in character between gametophyte and sporophyte. These were frequently observed to develop from the prothallia in the culture. On the surface of the prothallium, from which the single projection appeared, a number of antheridia had also been formed. The nature of the cells of the two generations is shown in a highly magnified portion of the region between the lamina of the leaf and the prothalloid part (*d*). There is also in this case a sharp line of demarcation between the two generations (fig. 3).

FIG. 3.—Cells of *Pteris sulcata* between gametophyte and sporophyte; ×170.

A number of forms were found in the culture which were intermediate in character, as shown by fig. 11. This growth resembled the leaf of an ordinary sporophyte. Typical epidermal cells including stomata, always present on the lamina of a leaf, were absent in this instance. No structure resembling a root was present. Fig. 4 shows the terminal portion of the lamina-like part.

An effort was made to study the nature of the development of the prothalloid portions from the earliest stages. The earliest stage found was one composed of 4 or 5 cells, formed in connection with the petiole of the primary leaf. For several days the growth was followed and during that time a number of cells had been produced, as shown in fig. 12. When the earliest stages were observed the leaf had already emerged from the prothallium, and distinctly gametophytic cells could then be seen. Since the number of instances of apospory appearing in the culture was not large, as compared with the number of sporophytes, a favorable opportunity to study the stages in sections was not offered. It is certain that such forms represented by figs. 8 and 16 were never wholly sporophytic and later became gametophytic. From such an instance as shown in fig. 5, however, it could readily be conceived that the prothallia developed from the primary leaf which was distinctly sporophytic in character.

FIG. 4.—Terminal portion of leaflike structure of *Pteris sulcata;* ×322.

It seems not improbable that the gametophytic cells were present at the earliest stages in the development of the embryo. Since the sporophyte of *Pteris sulcata* is of apogamous origin, there is an intimate connection between the cells of the gametophyte and the sporophyte. These cells may be carried upward by the sporophyte, and, retaining their power to divide, they may give rise to the gametophytic portions which have been described.

It has not so far been possible to state the conditions under which apospory occurred in the culture of the prothallia of *Pteris sulcata*. All attempts to induce the phenomenon have failed. Young sporophytes grown in subdued light produced no gametophytes aposporously. Portions of the leaves of young and old sporophytes when placed on moistened sphagnum also failed to develop prothallia.

The nuclear history of *Pteris sulcata* was not followed. It is very probable, from studies so far made, that there is no change in the chromosome number when the apogamous embryo originates. It is also likely that when the gametophyte is formed in connection with the embryo sporophyte there is no change in the chromosome number. On account of the limited number of aposporous developments in the culture no favorable opportunity was presented to count the chromosomes at a point in the life history when the gametophyte originates. It is believed, however, that the gametophyte thus produced and one formed by the germination of the spore have the same number of chromosomes.

The changes which are involved in the formation of an embryo of apogamous origin, except in the two *Lastrea pseudo-mas* varieties described by Farmer and Digby, are unknown. In these ferns, according to their description, fusion of adjacent prothallial cells and their nuclei initiate the formation of the embryo with the diploid number of chromosomes. It is certain from studies already made that such changes are not involved in any of the apogamous species which I have had an opportunity so far to investigate. Until the exact nature of the changes which are involved when the apogamous embryo originates are known, however, the origin of the aposporous developments in *Pteris sulcata* cannot be explained in a satisfactory manner.

Summary

1. The gametophyte generation of *Pteris sulcata* L. is ordinarily produced by the germination of a spore.

2. The embryo sporophyte is of apogamous origin.

3. The gametophyte generation of *Pteris sulcata* under certain conditions was produced aposporously.

4. The gametophytic portions or gametophytes were formed in connection with the lamina or the petiole of the primary leaf. In one instance a prothallium was produced from both lamina and petiole of the primary leaf. A sharp line of demarcation usually exists between the cells of the gametophyte and the sporophyte.

5. The prothallial portions developed antheridia, secondary prothallia, and in one instance a sporophyte-like outgrowth.

6. The antherozoids, produced by the aposporously developed prothallia, were actively motile and normal in appearance.

7. Occasionally forms intermediate in character between gametophyte and sporophyte were formed.

8. It seems probable that the origin of the aposporously produced gametophyte may be traced to an early stage in the development of the embryo. Since the embryo, on account of its apogamous origin, is intimately connected with the prothallium, it is not impossible that in some way cells of the prothallium may be embodied in the developing embryo. These cells, retaining the power to divide, may produce such outgrowths as have been described.

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EXPLANATION OF PLATES XVI, XVII

All of the drawings were made with the aid of a camera lucida. Figs. 5, 6, 7, 8, 11, 13, and 16 represent a magnification of about 30 times. All the other figures, with the exception of fig. 10, were drawn with a magnification of about 325. Fig. 10 represents a slightly greater magnification. The drawings were reduced one-half in reproduction.

PLATE XVI

FIG. 5.—Lamina of primary leaf of sporophyte of *Pteris sulcata* from both surfaces of which a number of prothallia appear as outgrowths of leaf blade; *a*, *a'*, antheridia.

FIG. 6.—Sporophyte of *Pteris sulcata* with large terminal prothallial portion formed in connection with primary leaf; *m*, *m'*, and *m''*, young secondary prothallia; *b* and *p*, prothallial portions; *s*, leaflike outgrowth.

FIG. 7.—Another sporophyte with large terminal prothallial portion; *c*, *d*, *e*, and *f* smaller prothallial portions; *n*, "light" area, cells being neither characteristically sporophyte nor gametophyte.

FIG. 8.—Sporophyte with well developed petiole of primary leaf; lamina displaced by prothalloid portion; *p*, old prothallium; *a*, antheridium.

FIG. 9.—Highly magnified portion of gametophytic region (*b*) and neighboring sporophyte cells shown in fig. 2.

FIG. 10.—Highly magnified portion of *n* and neighboring cells shown in fig. 3; chloroplasts are less numerous and smaller in paler region than in prothallial cells; cells of paler region are somewhat intermediate in form, partaking of nature of both generations.

FIG. 11.—Leaflike portion; epidermal cells of lamina not typically sporophyte, being regular in form and lacking stomata.

FIG. 12.—Early stage in development of prothallium (*p*) in connection with petiole of primary leaf.

PLATE XVII

FIG. 13.—Sporophyte with prothallium produced from both lamina and petiole of primary leaf; *o*, outgrowth from one surface of prothallium.

FIG. 14.—Highly magnified view of outgrowth (*o*) shown in fig. 9; cells in outgrowth much smaller than those of prothallium.

FIG. 15.—Three outgrowths (*o* and *s*) on other surface of prothallium represented in fig. 9; *r*, rootlike portion of outgrowth (*s*).

FIG. 16.—Primary leaf of sporophyte; *b* and *c*, prothallial portions; *m*, *m'*, secondary prothallia developed from dorsal surface of *b*; *p*, prothallium developed from petiole; *a*, antheridium.

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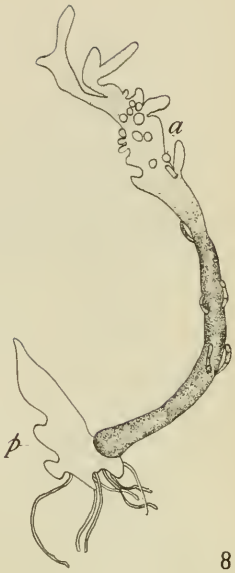

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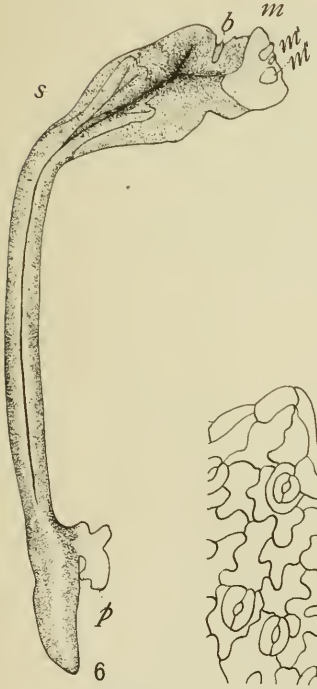

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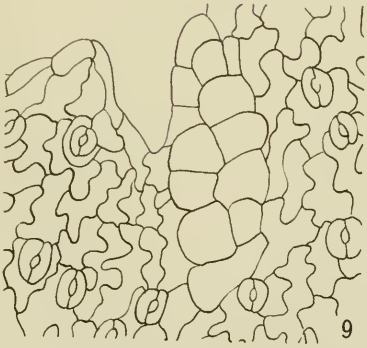

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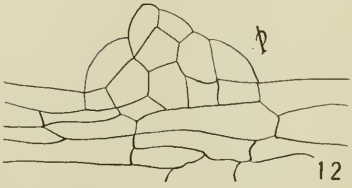

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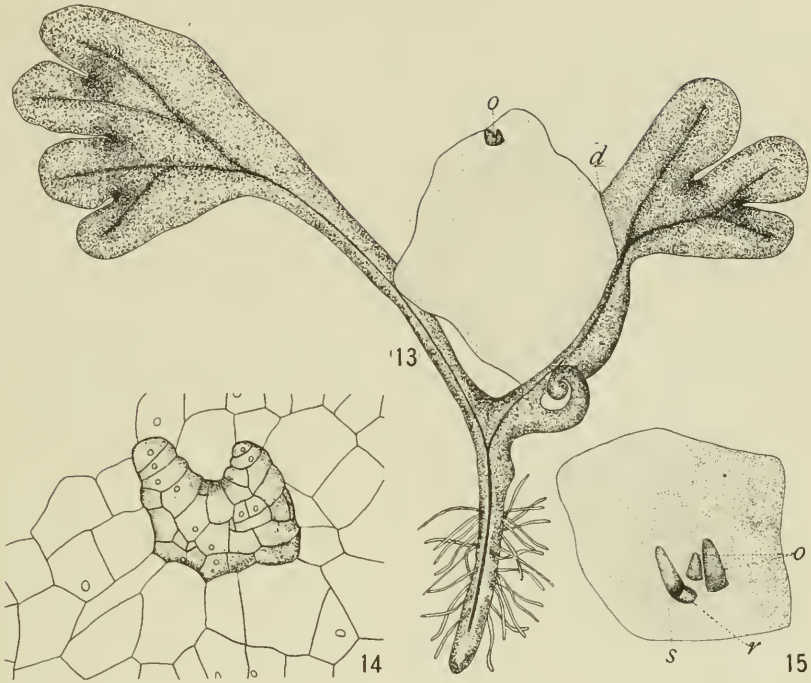
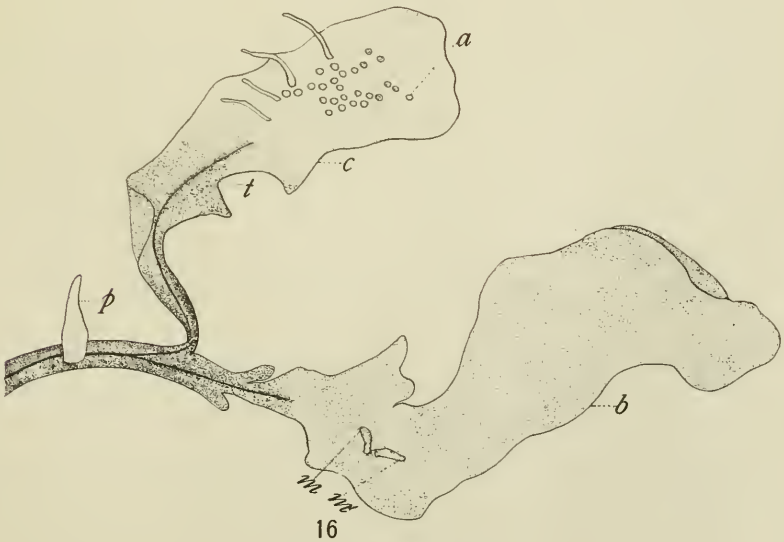
STEIL on PTERIS





STEIL on PTERIS

HYDROGEN CYANIDE FUMIGATION

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 249

E. E. Clayton

(WITH TWO FIGURES)

The object of this work was to secure data on the manner in which green plants are affected by exposure to hydrocyanic acid; with particular emphasis on the resistance of the plants to this gas and the modification of this resistance by various factors, external and internal. A number of articles have been published concerning the effect of cyanide on animals. More recently its action as an enzyme paralyzer has been brought forward prominently. Information as to the action of cyanide on plants is of scientific interest, and certainly of practical value, for hydrocyanic acid finds important use as an insecticide in orchard and greenhouse practice. Circumstances have forced the discontinuance of this work, with many phases of it incomplete, but enough facts have been established, and enough new lines suggested, to warrant its publication in this incomplete form.

Historical

Literature bearing on this problem is not abundant. The Department of Agriculture and several of the experiment stations, notably the California station, have published a number of bulletins dealing with fumigation as a commercial process; but the work done is of a kind which assists little in answering the fundamental physiological questions involved. The action of cyanide on the animal and in connection with various chemical processes has been thoroughly investigated, and from a consideration of these data we can gain much.

Schönbein (8) first called attention to the inhibitory effects of hydrocyanic acid. He worked with the leaves of plants, and also with animal blood, and found that the presence of the acid prevented each of these materials from decomposing hydrogen peroxide. He concluded that the extremely poisonous action of cyanide on the

animal was due to an incapacitation of the red blood corpuscles, which caused suffocation, a conclusion still generally accepted. GEPPERT (3), and still later VERNON (10), who worked with animals, found this same inhibitory effect of hydrocyanic acid on respiration. They showed that if a lethal dose has not been given the organism recovers completely; that is, if the organism is not killed it is not injured in any way. SCHROEDER (9), using the fungus *Aspergillus niger*, made a long series of determinations on the effect of potassium cyanide on respiration. He confirmed the previous work and emphasized the fact that, if cells were not exposed too long, recovery was complete. He also did some work with ether, and decided that the inhibition of respiration caused by treatment with this anesthetic was quite different in character from inhibition caused by treatment with cyanide. He characterized diminution of respiratory rate through treatment with ether as a secondary effect, brought about by previous death of the tissue; while diminution of respiratory rate through treatment with cyanide is a primary effect, the cyanide directly inhibiting respiration and killing the tissue.

MATHEWS (5) has offered strong arguments favoring a contrary view, namely, that hydrocyanic acid and anesthetics both act primarily on the respiratory processes, each affecting these in exactly the same manner.

Another activity of cyanide, which has come into prominence in recent years, is its rôle as an "enzyme paralyzer" and more specifically its ability to check certain chemical reactions carried on in the laboratory. BREDIG and IKEDA (1), also LOEVENHART and KASTLE (4), have found that in the catalytic decomposition of hydrogen peroxide the addition of a very small amount of potassium cyanide completely stopped the reaction. They concluded that the cyanide was acting on the catalytic agent. MATHEWS and WALKER (6), who worked on the spontaneous oxidation of a very reactive amino acid, cystein, found that a very small amount of potassium cyanide checked this action. Their statement of the probable explanation of this is interesting, since the same reasoning is equally applicable to other inhibitions of oxidation processes by cyanide. "The proportion of cystein molecules in a condition to be oxidized

at any given time is extremely small; while the proportion of active potassium cyanide molecules is large. The number of active oxygen atoms is also small. If we further assume that the cyanide unites with the cystein at the same point where the oxygen ordinarily combined, then the results obtained are easily understood." In plant respiration, with cyanide present, we have the same general condition; that is, the oxygen of the air is not able to oxidize the plant compounds, and we may suppose that the cyanide has acted in the same way. In the case of respiration, of course, we may have a chain of several oxidations and reductions, and the exact point at which the cyanide intervenes is not known.

Material

With the data at hand, while there is much on which to base inferences, there is nothing which tells of the behavior of an autotroph and the factors influencing this. Consequently, since this was pioneer work, it seemed highly desirable that it be done with normal green plants under normal conditions. The tomato was selected because it is easy to grow, sensitive to cyanide, and a plant commonly requiring fumigation in the greenhouse. Work under conditions such as those indicated is made difficult by the presence of factors not under control, which vary conditions with the different periods of time. In consequence of this it seemed best, in the case of most of the material experimented upon, not to try to carry on the work to its final expression, but merely to open up the way. The writer hopes at a later date to complete the work upon some of the more important phases with conditions carefully controlled.

Responses

The response of the plant to varying concentrations of hydrocyanic acid was observed as indicated by the subsequent growth of the plant. Two main points were considered in deciding the effect of a given concentration of gas: (1) the growth rate of the plants after fumigation; (2) the appearance and growth character of the plants after fumigation. The method was to select 7 or 8 groups of plants, similar in all respects, and to fumigate these on successive nights. Suitable checks were kept, and the growth of the treated

plants was compared with the growth of the check plants during the *same* days. Many series, made up as indicated, were run, the experiments covering a period of 5 months. It is obvious that the slow growing plants of January are not wholly comparable with the rapid growing plants of April. This matter cannot be controlled,

TABLE I

AVERAGE INCREASE OR DECREASE IN GROWTH DURING 12 DAYS FOLLOWING FUMIGATION

KCN per cubic feet	Plants measured	Percentage 1st to 4th days	Percentage 5th to 8th days	Percentage 9th to 12th days
0.001 gm.....	6	−16	+13	− 8
0.002 gm.....	10	−19.5	− 1.5	−11.5
0.003 gm.....	20	−11.5	−10.2	+ 9.3
0.004 gm.....	19	− 5.5	+21	− 7
0.005 gm.....	25	− 4.2	+11.4	+11.6
0.006 gm.....	30	− 5.7	+16	+13
0.007 gm.....	20	+17.7	+ 7	+ 6.5
0.008 gm.....	16	+33	+15.3	−18

TABLE II

PERCENTAGE OF 4 DAY PERIODS SHOWING DECREASED GROWTH RATE AND ALSO PERCENTAGE SHOWING INCREASED GROWTH RATE

KCN	Percentage showing rate decrease	Percentage showing rate increase
0.001 gm.....	66.6	33.3
0.002 gm.....	83.6	0
0.003 gm.....	63.5	36.4
0.004 gm.....	50.0	41.6
0.005 gm.....	33.3	66.6
0.006 gm.....	33.3	55.5
0.007 gm.....	16.6	75.0

and introduces an element of error. The time of exposure, length of exposure, temperature, and moisture conditions were similar in all cases. Using the growth of the check lots as 100 per cent, the loss or gain of the treated lots was computed, and from these values the averages shown in tables I and II were secured.

Consideration of these data shows that with concentrations of cyanide up to 0.004 gm. per cubic foot of air space, the effects were

somewhat detrimental to the growth of the plant. With 0.004, stimulation and retardation effects seem to be about balanced; but at 0.005, and continuing up through 0.006 and 0.007, stimulation effects plainly dominate. The plants giving these responses retained after the treatment all appearances of normal growth and metabolism.

With the concentration of 0.008 gm. KCN the first injury appeared, although there was some variation in this regard. Thus in one series no injury appeared before 0.01 gm. KCN. The harmful effect of this injury was very marked. Growth records were taken for concentrations up to 0.016, but unfortunately, with these damaging strengths (0.008–0.016), growth records do not always indicate the true condition of the plants. False stimulation

TABLE III

PERCENTAGE INCREASE OR DECREASE IN GROWTH FOLLOWING FUMIGATION

KCN per cubic foot	Percentage 1st to 4th day	Percentage 5th to 8th day	Percentage 9th to 12th day	Percentage 13th to 16th day
0.007 gm.: no injury	+14	+32	−33	−16
0.008 gm.: injury to parts of plants	+36	+44	−58	−44
0.009 gm.: injury to all plants	−37	+ 2	−63	−58
0.01 gm.	−27	−29	−66	
0.012 gm.	−16	−40	−12	

was frequently shown. This is an increase in growth rate of plants obviously in poor condition. The explanation of this false stimulation is that the growing tips of plants are rarely killed, and in consequence of the loss of considerable leaf area through injury these growing tips are forced into renewed activity. Thus badly injured plants in the course of a month's time form entire new tops, although old injured leaves drop off, for the most part. Usually, however, when injury occurred the depressing effects were so strong that this increased growth rate was very transient in character, or did not show at all, as is seen in table III.

A characteristic feature of this injury is the duration of the depressive effects. The plants were still far below normal at the

end of the 12 or 16 day period recorded. The following results were secured:

Gram KCN per cubic foot of air space	
0.001	
0.002	temporary depressive effects
0.003	
0.004	intermediate effects
0.005	
0.006	temporary stimulative effects
0.007	
0.008	relatively permanent depressive effects
0.009	
0.01	

It is interesting to note that the greenhouse white fly (*Aleyroides*) was killed at a concentration of 0.006 gm. KCN. Another point worthy of emphasis is that the actual difference in amount between a concentration of cyanide which brings about noticeable injury to the plants, and one which does no harm, may be very small indeed. This compares very well with previous statements (3, 9) that cyanide either killed or that recovery was complete.

There is not much work which can be cited in substantiation of the mixed stimulative and depressive effects found. In the work with hydrogen peroxide (4) very low concentrations of cyanide accelerated the reaction. Again it was discovered (7) that cyanide hastened the oxidation of an amino acid when the amino acid was present in an impure state. With these two exceptions the cyanide literature bearing on this work deals with inhibitory actions.

Factors

EXTERNAL FACTORS

Moisture.—In considering moisture as an external factor we deal only with the effect of free water on the leaf surface of the plants fumigated. It is common knowledge among greenhouse men that if plants go into the fumigation wet, considerable injury is often induced. In testing this I have found that some species are made more susceptible to injury by wetting the leaves, while other species are not visibly affected. The tomato is in this latter class. On closer observation it was found, in the case of the tomato, that

not only did the free water have no detrimental action, but actually, under certain conditions, gave beneficial results. This was true in the cases where the strength of fumigation was not sufficient to injure the plants (below 0.008 gm. KCN):

GROWTH DURING 12 DAYS FOLLOWING FUMIGATION

	Dry plants	Wet plants
Lot 1	100 per cent	127 per cent
Lot 2	100 per cent	115 per cent
Lot 3	100 per cent	139 per cent

With the following data, however, the fumigation was strong enough to injure the plants:

	Dry plants	Wet plants
Lot 1	100 per cent	115 per cent
Lot 2	100 per cent	99 per cent
Lot 3	100 per cent	100 per cent

Thus wetting the leaves had a beneficial effect if the fumigation was not strong enough to cause injury. With the appearance of injury this beneficial action ordinarily disappeared. The results just given are from experiments performed at wide intervals, and the plants used varied greatly as to size and age. Moisture present in pans as free water surfaces or saturated soil gave negative results.

TEMPERATURE.—The effect of temperature on plant resistance was determined qualitatively only. Excessive temperatures during the period of fumigation very materially increase the amount of injury. A variation of 20° F. has a marked effect; thus plants fumigated at 75° F. suffered much less injury than others which were fumigated at 95°. In what way temperature affects the plant, to cause these changes, has not been determined. One possible explanation is that the effect is through changes in permeability of protoplasm, such as RYSSELBERGHE, LEPESCHKIN, and ECKERSON have shown to occur, or even in permeability of wall structures. That it may be due to a change in the rate of chemical reaction is of course possible.

LIGHT.—Light undoubtedly exerts a direct action on plant resistance, but the conditions did not permit accurate observation on this point. Here permeability changes as well as catalytic effects of light appear possible. The indirect action of light as a regulator

of stomatal aperture and in connection with photosynthetic activity will be discussed later.

PLANT FACTORS

PROTECTIVE MEMBRANES.—Most plants which are highly resistant to cyanide are characterized by having thickly cutinized or suberized epidermal membranes which serve as a protection. As further evidence of the protective power of these, *Tradescantia zebrina* is made perceptibly more resistant by coating the upper surfaces of the leaves with Blackman's wax. The *Tradescantia* leaf has no stomata on the upper surface, and the reduction in injury must be due to the wax covering, thus making the thin epidermis relatively impervious. This increased resistance, when unobscured by a large amount of stomatal activity, is very marked. It is by no means possible to explain all differences in resistance on the basis of protective membranes, however. The radish endures without injury 3 times the strength of fumigation which a tomato endures, yet microchemical examination reveals but little difference in the cuticular development.

STOMATA.—The stomata seem to be the most important single factor in determining the amount of injury resulting from hydrocyanic acid fumigation. To ascertain the extent of their influence experiments were conducted in the following manner. Fumigations were run at various times of day and night, using like strengths of cyanide. Each lot of plants exposed included tomatoes and *Tradescantia zebrina*, the leaves of the latter being painted in various ways with Blackman's wax. After the beginning of each fumigation samples of epidermis were taken from several species with large stomata (*Geranium* and *Tradescantia*), and the amount of stomatal opening determined under the microscope.

FUMIGATIONS CONDUCTED ON A VERY DARK RAINY DAY (0.02 GM. KCN PER CUBIC FOOT)

EXPOSURE 1:30 TO 3:30 P.M.

Tomatoes	Badly injured
Tradescantia	
Leaves under surface coated	Uninjured (stomatal surface closed)
Leaves upper surface coated	Badly injured (stomatal surface open)
Leaves untreated	Killed (stomatal surface open)

Average stomatal opening 1:30 P.M., 3.5 μ

EXPOSURE 5:30 TO 7:30 P.M.

Tomatoes.....Slightly injured
Tradescantia
Leaves under surface coated..Uninjured (stomatal surface closed)
Leaves upper surface coated..Uninjured (stomatal surface open)
Leaves untreated.....Slight injury (stomatal surface open)
Average stomatal opening 5:30 P.M., almost all stomata were closed.

EXPOSURE 11:30 P.M. TO 1:30 A.M.

Tomatoes.....Occasional slight injury
Tradescantia
Leaves under surface coated..No injury (stomatal surface closed)
Leaves upper surface coated..No injury (stomatal surface open)
Leaves untreated.....No injury (stomatal surface open)
Average stomatal opening, none.

It was evident that the closing of the stomata greatly increased the resistance to fumigation. Approximately speaking, if 100 per cent were to represent the injury at 1:30 P.M., by 5:30 P.M. it had dropped to 10 per cent, and by 11:30 to 2 per cent. Why the plants were most resistant at the very late period was not entirely apparent, the stomata at 5:30 P.M. being, with very few exceptions, as tightly closed as at 11:30 P.M. Miss ECKERSON, however, has found that stomata in certain portions of some leaves lag behind as to closing time. During sunny weather there are great variations of light and temperature in the course of a day and night. This is in contrast with conditions during dark weather. In a sunny period, however, considerable work was done between 6:30 P.M. and midnight.

It was found that stomatal activity, as indicated by injury to the hypostomatous *Tradescantia*, continued on a gradually diminishing scale till 8:45 P.M. (about 2 hours after sundown). The stomata at this time were found to be almost closed. A noticeable fact was that at the later periods the correspondence between the amount of stomatal opening and the injury was not absolute. The injury at 8:00 P.M. was not so great as would have been expected from the size of the openings. In sunny weather, as in dark, there was a gradual increase in resistance of the plants during the night. The maximum of resistance was reached during the period between 11:30 P.M. and 1:00 A.M.

In conclusion it may be said that the amount of injury follows the stomatal movement rather closely. A fumigation at 5:30 P.M. on a dark day was about equivalent in injury to one at 8:30 P.M. on a bright day.

CHEMICAL FACTORS

WATER.—It was early observed that there were rather wide differences in the resistance of tomato plants grown under various conditions. It was found that when plants grew rather slowly, with a high chlorophyll content per unit area, they were very resistant to the hydrocyanic acid. Plants growing rapidly, with a low chlorophyll content per unit area, were very susceptible to injury from the hydrocyanic acid. Thus, judging solely by intensity of color, it was possible to select from a large group of plants 2 lots differing widely in their ability to withstand injury. Variations in water supply seemed to be the underlying cause of these differences, although other conditions will produce similar characters. To test this the following experiment was conducted: Twenty-four plants in vigorous growing condition were selected and divided into 2 lots. Lot 1 was watered only enough to keep growing, while lot 2 was watered abundantly. After 10 days, plants from lot 1, now dark green, were fumigated and found very resistant. Plants from lot 2, which were light green, were very easily injured by fumigation. An exhaustive chemical analysis of these plants was not made, but preliminary tests revealed one significant fact: the resistant plants (lot 1) had a greatly increased carbohydrate content. The reducing sugars claimed attention as being the most reactive of these substances, and also the ones most concerned in plant respiration. Determinations of the reducing sugar content of the leaves of lots 1 and 2 gave the following results (the samples were taken at 5:15 P.M.): lot 1, non-resistant leaves, 0.108 per cent calculated as dextrose per unit weight of green tissue; lot 2, resistant leaves, 0.57 per cent dextrose per unit weight of green tissue. Thus the resistant plants had much more reducing sugar. The actual amount is not large in either case, but the relative difference is great, lot 2 being 5 times as rich in reducing sugars as lot 1. The dry weight of the plants of lot 2 averaged little more than 1.5 times the dry weight of lot 1.

A possible rôle of reducing sugars as determiners of resistance was further tested by arbitrarily changing the sugar content of plants and observing the effects on resistance.

(1) Plants were placed in a dark box for 48–72 hours. This treatment brings the reducing sugars in the leaves practically to zero at the end of these periods. These plants, when fumigated, were found to be very easily injured.

(2) Plants treated exactly the same were taken from the dark box 12 hours before the fumigation and infiltrated with a glucose solution. They were returned immediately after the infiltration to the dark box, the exposure to light not exceeding 20 minutes. Plants thus made rich in glucose were highly resistant to cyanide injury. The following are extracts from data collected on this point:

FUMIGATION WITH 0.012 GM. KCN PER CUBIC FOOT, INFILTRATED AT 9:30 A.M. AT 6 CM. MERCURY PRESSURE

DARK BOX PLANTS	
Checks	Bad injury
INFILTRATED PLANTS	
0.25 per cent glucose	Slight injury
0.50 per cent glucose	No injury
1.00 per cent glucose	No injury (from fumigation)

FUMIGATION WITH 0.14 GM. KCN, INFILTRATED AT 10:00 A.M. AT 5 CM. MERCURY PRESSURE

DARK BOX PLANTS	
Checks	Very great injury
INFILTRATED PLANTS	
0.90 per cent glucose	No injury (from fumigation)
0.75 per cent glucose	No injury
0.60 per cent glucose	Very slight injury

With concentrations of glucose 0.9 per cent or above there was some injury to the plants from plasmolysis; such injury was distinct from that due to cyanide and caused no confusion. These experiments were repeated many times with uniformly decisive results. Frequently the check plants (not infiltrated) had their entire tops killed, while the treated plants showed little or no injury.

With these data in hand it is possible to say that for plants to be "normally" resistant they must have a fair content of reducing

sugar. MEYER has made determinations of the amounts of carbohydrates during the course of the day and night. Working with the leaves of *Tropaeolum*, he found that reducing sugars were at a minimum during the day but started to increase with nightfall. Analyses of tomato leaves yielded similar results; when the weather was bright the maximum reducing sugar content was found at 1:00–2:00 A.M. During dark weather this gradation was found to be much disturbed, as would be expected. The following figures were secured from the tomato leaves during such a period:

REDUCING SUGARS; WEATHER VERY DARK AND RAINY

1:30 P.M., 0.414 per cent, calculated as dextrose (net weight)
5:30 P.M., 0.335 per cent, calculated as dextrose
11:30 P.M., 0.285 per cent, calculated as dextrose

Presence of other factors made it impossible to ascertain what effect these variations had on resistance. There seems to be little doubt that the glucose in the plant acts as a protective agent against injury by cyanide. Considering glucose to have a direct effect, there are several possibilities concerning the manner of this action. (1) It may protect the plant by supplying an excess of molecules to unite with the cyanide entering. Cyanide does unite readily with glucose. (2) There is much evidence in physiological experimentation, with both plants and animals, showing that an excess of glucose present will temporarily take the place of missing oxygen. Asphyxiated animals produce glucose in excess amounts, other compounds being broken down. Plants, in absence of oxygen, behave normally for a time when glucose is supplied. It may very readily be that the protective action of the glucose is an indirect one, working through other channels. Thus it may possibly modify stomatal action.

What goes to make up a resistant plant, and under what conditions is it most resistant? We have given but little attention to the first of these questions; hence the broad problem of why certain species of plants are much more resistant to cyanide than others will be left without attempting an answer. Rather exhaustive comparative studies seem to be the only possible way of solving this.

Concerning the conditions under which plants are most resistant, the following statements are possible:

The resistance of the tomato to cyanide is increased by the presence of water on the leaf surface, but with some species wet leaves increase injury. Temperature should be moderately low. Light intensity should be low during the day preceding the fumigation. The plants should go into the fumigation with the stomata closed. A large amount of reducing sugars in the plant is correlated with maximum resistance. Checking growth by reducing water supply, during the period preceding the fumigation, increased resistance.

The action of cyanide on entering the plant is a very interesting question, and all evidence favors the conclusion that cyanide combines readily with the substances there. In certain species of plants cyanides in the form of glucosides occur, and on these naturally occurring cyanides a great deal of work has been done. The investigators (12, 13) are unanimous in considering that this cyanide is never in an uncombined state. Granting then that the hydrocyanic acid, on entering the plant, unites quickly with plant compounds, there may still be variations in the type of union. Thus possibly there may be adsorption under certain conditions and under other conditions chemical combination.

Methods for the determination of cyanide in plants have been devised (2, 11), but they are rather laborious. One method is based on the fact that a cyanide in a picric acid–sodium carbonate solution gives a red color. The depth of the color, as compared with a standard range, is the basis of estimation. The coloration is due to the strong reducing action of the cyanide, but unfortunately the cyanide is not the only substance present in the plant which gives the reaction. For accurate quantitative work it is necessary to isolate the cyanide compounds. This was not done at this time; instead, estimations were made on unit weights of leaf tissue. The leaves were put in the picric acid–sodium carbonate solution, and observations were made after 24 hours by means of a colorimeter. The phrase "reducing substance content" is used because, as already stated, the cyanides are not the only things reacting. The variation in values secured, during the course of a series of

fumigations, is very interesting. In the data T represents an empirical measure.

Fumigation strength in gm. KCN per cubic foot	Reducing substance content
0.003	T 7.5
0.004	T 8
0.005	T 8
0.006	T 8.5
0.007	T 9
0.008 point of first injury	T 4
0.009	T 3.5
0.010	T 3.5
0.012	T 2.5
0.016	T 2
0.020	T 1.5

The sudden drop at the point of first injury was very noticeable; thus from T 9 to a value less than half. Considering this drop as an index of chemical changes, it agrees well with the results of the growth experiments recorded at the beginning of the paper. Every indication was found, also, that the action of the cyanide, at a concentration just below the point of external injury, was radically different from its action at and beyond this point.

There are two main types of injury resulting from cyanide fumigation: (1) the killing of definite areas of leaf and, much more rarely, stem tissue. This injury is always localized on the younger portions of the plant. (2) Injury in the form of an epinastic response. This is frequently found in cases where the fumigation was just a trifle too strong. As a rule, under these circumstances, there is no apparent injury to the plants for a period of 5–7 days after the fumigation, and then a twisting of certain leaves (epinasty) becomes apparent. This kind of injury is quite distinct from the distortion which arises through an excessive killing of tissue.

The drawings in fig. 1 were secured after fumigating, with a damaging strength, a house containing tomato plants of different ages. The movement of the injury from the inner portions of the leaf in the old plants to the outer portions of the leaf in young plants was noticeable. The drawings show all the injured leaves from representative plants. Even with the very large plants the injury did not extend below the third or fourth leaf from the tip.

FIG. 1.—Injury from cyanide fumigation to tomato plants ranging in age from seedlings to mature plants (*a*, *b*, *c*, *d*); the injury is shown by the black areas, and it will be noted that the leaf tissues killed in the small seedlings (*a*) are peripheral, and also more or less apical.

There are several possible explanations of this localization on the younger portions. The epidermal membranes are not so well developed in the young leaves and it is easier for the cyanide to enter. The younger and more rapidly growing portions of a plant are the first to suffer in absence of oxygen. It may be that the very reactive state of the protoplasm in these parts makes them less resistant.

FIG. 2.—Older plants, in which there is a steady change in the location of the injured areas, until with old bearing plants the killed area is basal, and is not limited to the margin of the leaf.

The rapidity with which cyanide can kill the plant tissue is another point of interest. The plant can live in the total absence of atmospheric oxygen for some time. However, 5 hours after the beginning of a damaging fumigation it was possible to see the dead leaf areas, dark and water-soaked in appearance.

It may be said that there are several things which stand out prominently as causes for the unsatisfactory results often secured from greenhouse fumigation with cyanide. First, there is a lack of

appreciation of the necessity for delicate regulation of amounts of cyanide. Every separate greenhouse varies in its ability to retain the gas, and it is not alone the amount of cyanide which is put in that counts, but rather the amount which is retained. Thus definite recommendations cannot be given more than it is possible to give a universal fertilizer formula. Second, it is the usual thing to start the fumigation during the latter part of the afternoon when the stomata are still open. This is sure to induce excessive injury. It is safe to start fumigation 2.5–3 hours after sundown on a bright day, or at sunset on a very dark day.

Summary

1. Different concentrations of hydrocyanic acid gas gave effects ranging from stimulative to depressive. The maximum of beneficial results was secured from concentrations deadly to insect life, but just a little below the point of first injury to the plant.

2. External factors having important action on the resistance are as follows: (*a*) wetting the leaves had a beneficial effect on the tomato; (*b*) reduced temperature and low light intensity during the day preceding fumigation increased resistance.

3. Injury closely paralleled the stomatal movement, increasing as the size of stomatal aperture increased.

4. A higher or lower water supply in the soil affected resistance, through hastening or retarding the growth rate. Rapid growing plants were susceptible to injury, while slow growing plants were more resistant.

5. High reducing sugar content seemed to be correlated with maximum resistance.

I wish to acknowledge the assistance given by Dr. William Crocker and Dr. Sophia H. Eckerson, under whose direction this work was conducted.

University of Wisconsin
Madison, Wis.

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STUDIES OF SOME PORTO RICAN FUNGI

Leo R. Tehon

(with plate xviii)

The fungi reported and described in this paper represent a miscellaneous group of specimens from the collections of Dr. F. L. Stevens in Porto Rico. They were selected by him and turned over to the author for study. Most of the numbers present points of special interest to the mycologist, as will be seen later in connection with the individual species. Type and co-type specimens are deposited in the herbarium of the University of Illinois, and in the herbarium of the New York Botanical Garden.

Coccomyces De Notaris

Coccomyces clusiae (Lev.) Sacc. Syll. Fung. 8:147. 1889.

On dead leaves of *Clusia rosea* Jacq., Maricao, July 19, 1915. nos. 882a, 8765 (figs. 8, 9, 10).

It is assumed that these specimens belong to this species. No exsiccati have been available for comparison and the descriptions extant are too meager to allow of a positive determination. The fungus, however, clearly belongs to the genus *Coccomyces*, and its occurrence on *Clusia* is regarded as being sufficient to warrant calling the specimens *C. clusiae*. For the convenience of other workers an emended description of the species follows:

Spots irregular, large, light-colored, amphigenous, thickly dotted with ascomata. Ascomata amphigenous, but chiefly hypophyllous, black, circular, subepidermal, erumpent, 0.5–2 mm. in diameter, rupturing with the epidermis by 4 or 5 radial splits. Paraphyses filiform, numerous, coalescing above into a brown epithecium. Asci long, narrow, cylindrical, 100–135×7 μ, 8-spored; ascospores filiform, 1 μ in diameter and nearly as long as the asci, multicellular, hyaline.

Under the microscope the bottoms of empty ascomata present a curious, honeycombed appearance as the result of numerous pits in the hymenial layer (fig. 9). With the hope of finding an explanation for the pitted condition there found, microtome sections were made of the ascomata, some of which contained

asci and some of which did not. An examination of the sections disclosed the fact that, in certain regions of the hymenium, ascigerous hyphae do not develop. In these places there is, therefore, only a very thin covering formed over the bottom of the ascoma and a corresponding pit in the hymenial surface results. The formation of a large number of these pits causes the honeycombed appearance on the floor of older ascomata from which the asci have disappeared (figs. 9, 10).

COCCOMYCES MUSAE (Lev.) Sacc. Syll. Fung. 8:752. 1889.

On dead leaves of a cultivated species of *Musa*, Rio Maricao, above Maricao, September 10, 1913, no. 3631 (fig. 7).

With this, as with the preceding species, no exsiccati have been available for comparison, and the current descriptions are not such as would make possible an accurate determination. The fungus clearly belongs to the genus *Coccomyces*, however, and its occurrence on *Musa* is regarded as being sufficient, with other general superficial characters, to place it under the species already described for that host. Since, however, the previous description is so meager, an emended specific description is here given.

Spots amphigenous, whitish or straw-colored, roundish, 5–10 mm. in diameter, frequently confluent, uniformly but sparsely dotted with ascomata. Ascomata punctiform, black, 350–750 μ in diameter, rupturing irregularly by a 3 or 4-partite radial cleft. Asci cylindrical, short-stipitate, 55–90×10–12 μ, 8-spored; ascospores long, rodlike, with obtuse ends, 50–70×3 μ, hyaline and multiseptate at maturity. Paraphyses long, filamentous, numerous, exceeding the asci, hyaline.

MELIOLA Fries

The following species constitute a few that were overlooked, either because of their resemblance to other fungi, or because of their inevidence on a host collected for the sake of other fungi upon it, when the monograph of Porto Rican Meliolas was compiled by Dr. STEVENS.[1]

Meliola conferta, sp. nov.—Spots amphigenous, irregularly circular, punctiform, 0.5–1 mm. in diameter. Mycelium brown, densely compacted, radiate, branches opposite, filaments 8 μ in diameter.

[1] STEVENS, F. L., The genus *Meliola* in Porto Rico. Ill. Biol. Monographs 2: no. 4. 1916.

Capitate hyphopodia opposite, exceedingly crowded, intricately interlocked with the hyphopodia of adjacent filaments, apical cell ovoid to globular, 12×18 μ in diameter, basal cell 3–6 μ long. Mucronate hyphopodia few, opposite, situated mostly near the ends of the hyphae, bottle-shaped, 18 μ long. Mycelial setae none; perithecial setae few, 6–8, arising subapically, straight, 7×80 μ, tips obtuse.

Perithecia 120–135 μ in diameter, rough; asci 2-spored, soon evanescent; ascospores brown, 4-septate, obtuse, slightly constricted, 40×15 μ.

On leaves of *Rhacoma crossopetalum* L., Mona Island, December 20, 1913, no. 6147 (type) (figs. 17, 18, 19).

The compact habit of this species is very remarkable. Starting from a single spore, the spot develops into an exceedingly thick black mass. The hyphae are arranged radially and branch repeatedly, so that when the whole colony is seen under the microscope it is almost impossible to distinguish individual filaments. The radial habit, when the fungus is viewed in place on the host leaf, strongly suggests the radial character of an immensely large Microthyriaceous perithecium.

This *Meliola* is probably most closely allied to *M. parethesicola* Stevens, but can readily be separated from it because of its denser habit and the fact that the perithecial setae arise subapically instead of basally. What appears to be a constant manner of spore germination has repeatedly been seen. From one of the end cells of the spore there is put out a capitate hyphopodium (fig. 18) which acts as an anchor for the spore. Following this, filaments are sent out from the other cells of the spore and begin to branch and rebranch immediately. Thus the characteristic compactness of growth is assumed almost with the germination of the spore.

MELIOLA PEREXIGUA Gaill. Le Genre *Meliola*. 98. 1892.

On leaves of *Petiveria alliacea* L. at Corozal, February 21, 1913, no. 415.

This species has not hitherto been reported from Porto Rico, or on this host. The specimen, however, agrees thoroughly with GAILLARD's description of the species.

MELIOLA ASTERINOIDES Wint. Hedwigia 96. 1886.

On the upper surfaces of leaves of *Genipa americana* L., no. 7135 (figs. 11–16).

This specimen is referred to this species with some hesitation. The description given by GAILLARD[2] characterizes the fungus as forming very small colonies, 0.25–1 mm. in diameter, with only a small number of subdimidiate

[2] GAILLARD, A., Le Genre *Meliola*. 58. 1892.

perithecia (often only one) near the center of the spot, each of which sends out long radial filaments. Although no exsiccati having been available for comparison, it was impossible to be certain of a correct interpretation of the description, our specimen seems to show certain more or less inconformable characters.

The colonies are large, 1–5 mm. in diameter, and give rise to a comparatively large number of perithecia scattered uniformly throughout the entire colony. The perithecia are dimidiate, rather than subdimidiate, and are made up of radiating hyphae which extend somewhat beyond the limits of the perithecium to form an areola (fig. 16) somewhat similar to, but entirely distinct in aspect from that surrounding the perithecia of *M. aibonitensis* Stevens, *M. comocladiae* Stevens, etc.

GAILLARD called attention to the Microthyriaceous aspect of the perithecia of *M. asterinoides*, but the present specimen may be seen actually to possess Microthyriaceous characters in its dimidiate and radially formed perithecium which opens by a false ostiole. It is possible, therefore, that the specimen may throw some light upon the phylogenetic relationship of the genus.

In mycelium and spores, this specimen is characteristically a *Meliola;* but in perithecial development and characters it is almost typically Microthyriaceous. The development of the perithecium begins by the extrusion of a hyphopodium-like branch, 1 or 2 cells long, at some point, usually near the growing tip (fig. 12) of a filament. The tip of this special branch then becomes swollen (fig. 11*a*), and just beneath the swollen part a small thumblike projection (fig. 11*b*) is sent out in such a manner that, at its completion, what may be termed the first stage resembles somewhat the profile of a closed fist. The thumblike portion now grows until it reaches approximately two-thirds the size of the enlarged tip, and lies alongside it (fig. 12*a*). There now appear, along the outer edges of the 2 prongs thus formed, evident indentations (fig. 13*a*) that eventually cut the tip of the perithecial branch into 5 or 6 cells, which may be regarded as perithecial mother cells. Simultaneously with the appearance of the marginal indentations, one finds growing out radially from beneath the edges of the perithecial mother cells a number of rather light colored hyphae (fig. 13*b*) which, as growth continues, eventually form a complete circle of elongated radiating cells (fig. 14*a*) about the cells from which they originated. When

the circle has reached full development most of its cells will be found to possess terminal indentations (fig. 14*b*), indicative of a dichotomous scheme of branching, and from beneath the outer edges of some cells may already be seen (fig. 14*c*) the beginning of a new circle similar to the last.

Thus by the addition outwardly of circle after circle of radiating, dichotomously branching cells (figs. 15, 16) the complete perithecium is formed. Fig. 16 shows a mature perithecium illustrating the true dimidiate character. On ripening, the central portion becomes black and opaque, so that it is impossible to see the hyphal structure, but around the edge there is always apparent the areola of radiating close lying hyphae, some of which extend outward rather farther than the others and are capped by a hyphopodium-like head cell (fig. 16*a*). In some cases these elongated hyphae from the perithecial areola may even send out capitate hyphopodia laterally.

The false ostiole is a character which, if taken together with the dimidiate character of the perithecium, may also be of some phylogenetic significance.

Although *Meliola* has never been definitely allied to the Microthyriaceae, ENGLER referring it to the Aspergillaceae and SACCARDO to the Perisporiaceae, the impression is becoming more and more firmly rooted that *Meliola* is closely related to the Microthyriaceae. In this connection, the present specimen shows certain characters which strengthen that impression. The specimen may even be regarded as a transitional stage connecting this genus with the typical genera of the Microthyriaceae.

Meliola cestri, sp. nov.—Colonies epiphyllous, irregularly circular, 1–3 mm. in diameter. Mycelium dark, straight, forming a rather close network, filaments 9–10 μ in diameter.

Capitate hyphopodia alternate, 35 μ distant, head-cell cylindrical to globose, 16–20×8–13 μ; basal cell 4–5 μ long. Mucronate hyphopodia opposite, bottle-shaped, 24–28×9 μ. Perithecial setae none; mycelial setae numerous, straight, black, 650–850×10–11 μ, tips obtuse.

Perithecia numerous, grouped in the center of the colony, surrounded by an areola of hyphae when young, smooth, 225–275 μ

in diameter. Asci soon evanescent; ascospores 4-septate, dark brown, cylindrical, 50–55×18–20 μ, definitely constricted at the septa.

On leaves of *Cestrum* species, Mayaguez, June 29, 1915, no. 7576 (type).

This species is entirely distinct from *M. gesnerii* Stevens, which has also been reported on this host.

MELIOLA CLUSIAE Stevens, Ill. Biol. Monographs 2:75. 1916.

On *Clusia gundlachi* Stahl, El Alto de la Bandera, July 15, 1915, no. 8670. Previously reported on *Clusia rosea* Jacq.

Meliola bayamonensis, sp. nov.—Colonies hypophyllous, 2–5 mm. in diameter, mycelium a very loose network of threads; branches alternate, hyphae dark, 4 μ in diameter, wavy.

Capitate hyphopodia alternate, 30–60 μ distant, the head cell globose to oval, 8–10 μ in diameter; the basal cell variable, 6–16 μ long. Mucronate hyphopodia few, alternate, 14 μ long. Mycelial setae none; perithecial setae 5–7, arising basally, decumbent, dark brown to opaque, 225×4–5 μ, apex acute.

Perithecia scattered, 100–135 μ in diameter, rough. Asci soon evanescent, ascospores 4-septate, 27–30×7 μ, slightly constricted at the septa.

On *Psychotria pubescens* Sw., at Bayamon, February 19, 1913, no. 392 (type).

This species is separated immediately from the variety described on the same host by STEVENS as *M. glabra.* var. *psychotriae* by the presence of perithecial setae. It is also distinct from *M. psychotriae* Earle on account of its remarkably looser habit, the characteristic waviness of the mycelium, its lighter color, the shape of the capitate hyphopodia, and the strikingly short basal cell.

Meliola marcgraviae, sp. nov.—Colonies epiphyllous, irregular, 3–10 mm. in diameter, branching opposite, hyphae dark to opaque, 5–6 μ in diameter.

Capitate hyphopodia alternate, 32 μ distant, head-cell globose, 11 μ in diameter; basal cell short, 5 μ long. Mucronate hyphopodia mostly opposite, but frequently alternate, flask-shaped, 12–14 μ long. Mycelial and perithecial setae none.

Perithecia scattered, small, 65–75 μ in diameter. Asci soon evanescent, ascospores 4-septate, light brown, cylindrical, slightly constricted, 40×15 μ.

On leaves of *Marcgravia rectiflora* Tr. and Planch., Porto Rico, July 16, 1915, no. 8722 (type).

The colonies formed by this *Meliola* are so inconspicuous as to pass entirely unnoticed. It is only when the leaf is placed under a lens that the colony is to be seen.

PHYLLACHORA Nits.

Phyllachora quadraspora, sp. nov.—Stroma variously shaped, mostly oval to linear, 0.5–1×0.5 mm., with epidermal clypeus on either side of the leaf usually bilocular. Locules sub-spherical, 115–125 μ in diameter. Asci cylindrical, short-stipitate, 100–110 ×10–12 μ, 4-spored; spores hyaline, granular, 1-guttulate, elliptical, 20–22×8 μ. Paraphyses present.

On leaves of *Paspalum glabrum* Poir, Maricao, no. 8803 (type); *P. conjugatum* Bergius, Tanama River, no. 7856 (fig. 4).

In naming this species the author has endeavored to follow the monograph of the genus by THEISSEN and SYDOW.[3] The fact that the stroma is laid down in the mesophyll of the leaf brings the fungus into the genus *Phyllachora;* but the occurrence of only 4 spores in the ascus would seem, on the other hand, to exclude it. This, however, need not be true, since THEISSEN, in characterizing *Ph. graminis* as having spores up to 8 ("zu acht") leaves the implication that the spore number may be fewer than 8.

PHYLLACHORA GRAMINIS (Pers.) Fcl. Symb. Myc. 218. 1869.

This species has previously been reported by GARMAN on an undetermined species of *Paspalum* from Sabana Grande in 1915. It is also to be noted on *Paspalum glabrum* Poir., at Rosario, no. 9495a.

Phyllachora ischmaemi, sp. nov.—Stromata appearing on the upper side of the leaf, circular, crowded, and often confluent, laid down in the mesophyll, 0.75–1.25 mm. in diameter. Clypeus 24–26 μ thick. Locules 2-several in a stroma, spherical to flask-shaped, 125–145 μ in diameter. Asci cylindric to clavate, 105–150×10–12 μ, 8-spored. Spores hyaline, spherical, uniseriate, 8 μ in diameter, with a single guttula. Paraphyses present, filiform.

On leaves of *Ischmaemum latifolium*, St. Pierre, Martinique Island, no. 2972 (type) (figs. 2, 3).

[3] THEISSEN, F., and SYDOW, H., Die Dothidiales. Ann. Mycologici 13:437. 1915.

STIGMATEA Fries

Stigmatea guettardae, sp. nov.—Spots large, 0.5–3 cm. in diameter, irregular, light brown or red in young stages and ashy or white in old stages, always sharply defined by a dark brown border.

Perithecia epiphyllous, gregarious, grouped in the center of the spot, small, black, 60–80 μ in diameter. Ostiole erumpent, small, 6–10 μ in diameter. Asci obliquely oblong, short and abruptly stipitate, obtuse above, 25–40×8–11 μ, 8-spored. Spores long-elliptic, 2-celled, the septum at or nearly at the center, slightly constricted, hyaline, 11–13×2–3 μ. Paraphyses present, long, filiform, septate, hyaline to yellowish.

On *Guettarda ovalifolia* Urb., Maricao, January 10, 1913, no. 191 (type); Barros, January 2, 1913, no. 164; Maricao, April 5, 1913, no. 771; July 19, 1915, no. 8804; Monte Alegrillo, no. 4741; Indiera Fria, Maricao, October 8, 1913, no. 3338.

On *Guettarda scabra* (L.) Lam., Tanama River, July 6, 1915, no. 7851.

PHAEOSPHAERELLA Karst.

Phaeosphaerella paspali, sp. nov.—Perithecia amphigenous, sunken, sub-spherical, 125–137 μ in diameter; ostiole minute, 10–15 μ in diameter. Asci crowded, sub-cylindric, 55–60×8 μ, 8-spored; spores brown, oblong to fusoid, unequally 1-septate, slightly constricted at the septum, 12–15×3–5 μ. Paraphyses none.

On leaves of *Paspalum glabrum* Poir., Maricao, no. 8803a (type) (fig. 5).

CONIOTHYRIUM Corda

Coniothyrium marisci, sp. nov.—Spots oval to linear, yellowish, with a dark brown margin. Pycnidia gregarious, amphigenous, sub-spherical, 120–130 μ in diameter, subepidermal, only the ostiole erumpent. Perithecial wall thick (35 μ); ostiole 16–20 μ in diameter. Spores dark brown, ellipsoid or globose, 5–7×2.5 μ. Conidiophores not apparent.

On *Mariscus jamaicensis* (Crantz) But., no. 124 (type) (fig. 6).

PESTALOZZIA De Notaris

Pestalozzia lucumae, sp. nov.—Spots epiphyllous, black, irregular, 2–5 mm. in diameter. Acervuli subepidermal, erumpent,

white or ashy at maturity, 0.5–2 mm. in diameter, crowded on the stroma, circular or elongate, and rupturing irregularly. Conidia elliptic or slightly falcate, 14–18×4–5 μ, 4-septate, slightly constricted; central cells fuscous, end cells hyaline, the apical cell conic, ornamented by 2 widely divergent, hyaline, filiform setae 7–10 μ long, basal cell prolonged into a single hyaline seta 3–5 μ in length.

On leaves of *Lucuma multiflora* A. DC., Monte Alegrillo, July 20, 1913, no. 2301 (type).

It was a surprise to find this specimen a *Pestalozzia*. Its superficial characters suggest strongly a Phacidiaceous form, but a close examination reveals the fact that instead of an ascoma, the fruiting body is an acervulus. The acervuli are grouped closely on a large stroma which causes a characteristic black spot on the leaf.

Helminthosporium Link

Helminthosporium folliculatum Corda var. brevipilum Corda, Icon. 2:13, 1838.

On *Paspalum conjugatum* Bergius, Tanama River, July 6, 1915.

Acrothecium Preuss.

Acrothecium flacatum, sp. nov.—Mycelium light olivaceous, septate, 2.5 μ in diameter, attacking the spikelets and thickly covering the glumes and awns of the flowering heads. Conidiophores dark olivaceous in color, straight, somewhat bulbous at the base, 96–125×5 μ. Spores olivaceous, luniform, 3 or 4-septate, borne apically on the conidiophores in fascicles of 3–5, 35×10 μ; central cell very much enlarged, dark, and not equilateral; the terminal cells small and nearly hyaline.

On *Setaria* species, Porto Rico, 1915, no. 9181 (type) (fig. 1*a*, *b*, *c*).

Another fungus belonging to this genus and possessing the same strikingly characteristic spore is generally reported as the cause of the "ringspot" of sugar cane. It was first mentioned by Breda de Haan in 1892, and was thought by him to be the conidial stage of *Leptosphaeria sacchari*. In 1898 a fungus identical with that of Breda de Haan's was described by Wakker[4] on dead leaves of sugar cane under the name of *Acrothecium lunatum*.

The present species, however, is clearly distinct from that described by Wakker, in that the conidia are more frequently 4-septate than 3-septate, distinctly larger, and regularly borne in fascicles of 3–5 on the tips of the

[4] Wakker, J. H., and Went, F. A. F. C., De Ziekten van het Suikerriet op Java. pp. 149, 196. Leiden. 1898.

conidiophores. Cultures of *Acrothecium lunatum* frequently show a secondary group of spores borne below the apical group. Furthermore, the 2 hosts being so widely separated phylogenetically, it is probable that they would not be attacked by the same parasite.

Cercospora Fries

Cercospora personata (B. and C.) Ellis, Jour. Mycol. 1885.

On the leaves of *Arachis hypogaea* L., Trujillo Alto, August 17, 1915, nos. 2506, 2447.

These specimens agree in general with the species, comparison having been made with reliable exsiccati. They do present a peculiarity, however, in that the spots are consistently smaller, and much more regularly circular in outline.

Trichostroma Corda

Trichostroma axonopi, sp. nov.—Spots oval to linear, yellowish with a definite brownish or purple border. Sporodochia gregarious, black, globular to oval, verruciform, often confluent, 95–125 μ long. Setae black, straight or the tips sometimes repand, rigid, base bulbous, 65–85 μ long, few-septate. Conidia brown, globular to ovoid, 5 μ in diameter.

On leaves of *Axonopus compressus* (Sw.) Beauv., College grounds, Mayaguez, May 30, 1913, no. 924 (type).

EXPLANATION OF PLATE XVIII

Fig. 1.—*Acrothecium falcatum:* conidiophore and conidia (*a*, *b*, *c*).

Fig. 2.—*Phyllachora ischmaemi:* section of stroma.

Fig. 3.—*Phyllachora ischmaemi:* ascus.

Fig. 4.—*Phyllachora quadraspora:* ascus.

Fig. 5.—*Phaeosphaerella paspali:* ascus.

Fig. 6.—*Coniothyrium marisci:* section of pycnidium.

Fig. 7.—*Coccomyces musae:* ascus and ascospores.

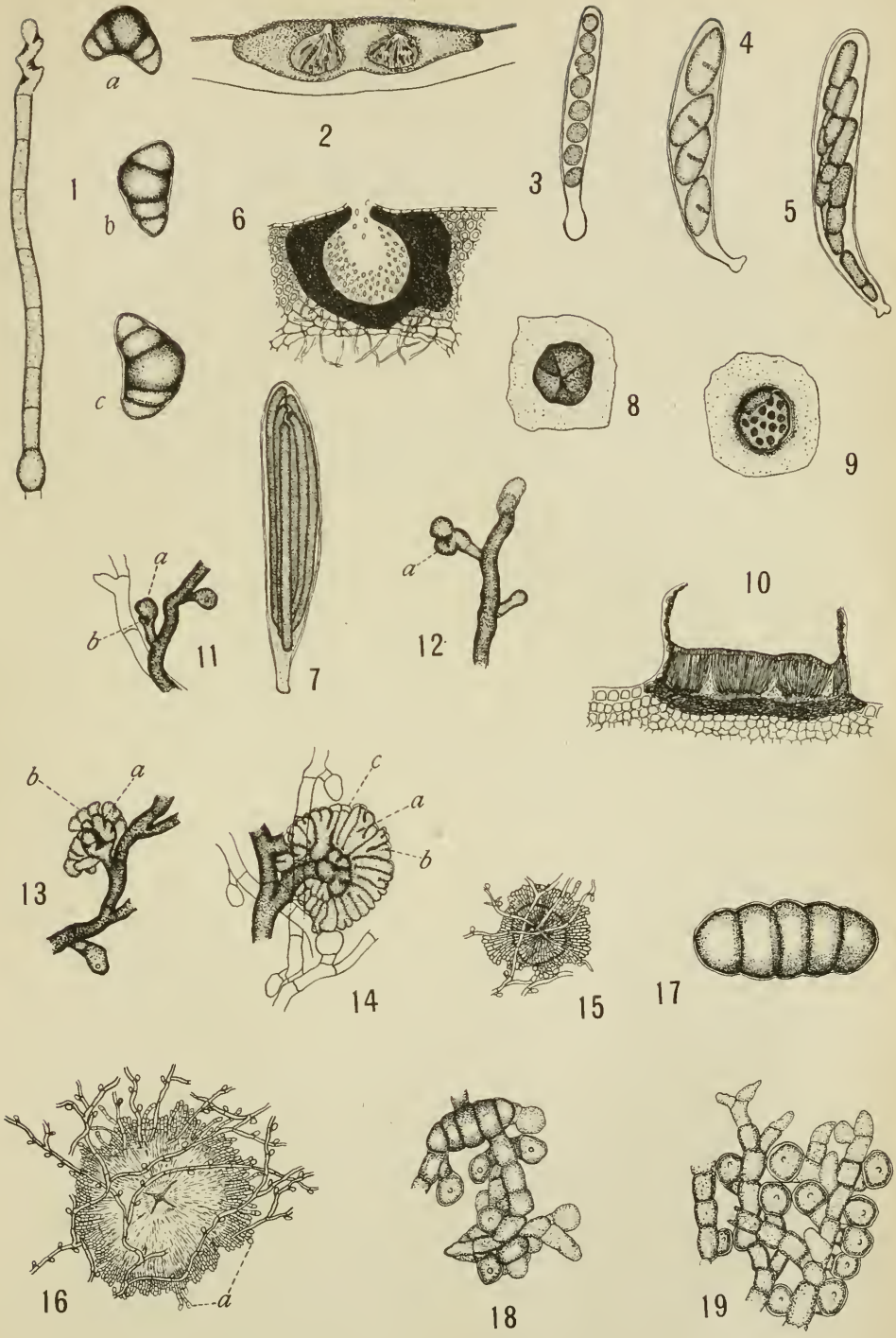
Fig. 8.—*Coccomyces clusiae:* habit sketch of single ascoma.

Fig. 9.—*Coccomyces clusiae:* old and empty ascoma showing honeycombed appearance of floor.

Fig. 10.—*Coccomyces clusiae:* cross-section of ascoma showing localization of ascus producing hymenium which results in honeycombed appearance seen in fig. 9.

Figs. 11–16.—*Meliola asterinoides.*

Fig. 11.—Beginning of perithecium: *a*, large swollen tip; *b*, smaller thumblike projection.



TEHON on PORTO RICAN FUNGI

FIG. 12.—Further stage in perithecial development: *a*, thumblike projection grown to approximately two-thirds size of swollen tip.

FIG. 13.—Third stage: *a*, indentations appearing on margins of the 2 prongs; *b*, radial hyphae developing from under side of perithecial mother cells.

FIG. 14.—First circle of radial cells complete: *a*, radial cells; *b*, marginal indentations; *c*, new cells which will form another concentric circle outside first.

FIG. 15.—Perithecial development in intermediate stage: pronounced radial character evident.

FIG. 16.—Mature perithecium: showing areola of radiating hyphae, dimidiate character, and false ostiole; *a*, elongated perithecial hyphae capped by hyphopodium-like cell.

FIGS. 17–19.—*Meliola conferta*.

FIG. 17.—Ascospore.

FIG. 18.—Ascospore germination: showing manner in which compact habit is assumed.

FIG. 19.—Portion of mycelium illustrating compact habit.

CURRENT LITERATURE

BOOK REVIEWS

The living cycads

In a recent volume of the "University of Chicago Science Series" CHAMBERLAIN[1] presents in untechnical language some of the results of his 15 years of study on cycads. This group of 9 genera has several advantages from the point of view of popular treatment, and of these the writer makes good use. It may safely be said that no one is so well fitted as the author to deal with this subject, because he approaches the task not only as an expert student of the group, but also as one who knows the cycads as living plants, on account of his extended travel in regions where cycads occur. The title of the book was selected, so the author tells us, to contrast with WIELAND'S *The fossil cycads.*

The account is divided into 3 sections, part I dealing with the collecting of the material, part II with the life history, and part III with the evolution and phylogeny of the group. The first part recounts the author's journeyings in the 3 cycad regions, tropical America, Australia, and South Africa, but is much more than a narrative, for it includes important observations taken in the field which help to clear up certain disputed points. This part is rendered attractive by the author's excellent photographs of field specimens as well as by his graphic style, and will probably be the most interesting section to the non-botanical reader. The section on life history includes a short chapter on vegetative structures, in which the vascular tissues receive scant attention. The very significant anatomical features were probably considered too technical for presentation to a general audience. The sporogenous and gametophytic structures, which have attracted so much attention to the group, are clearly described and figured, and are compared with the corresponding structures of ferns. It is in this part of the book that the author's particular contribution to our knowledge of cycads comes out conspicuously. One realizes how many gaps in the story have been filled during the past decade. Part III, the shortest in the book, passes on from fact to speculation, and brings the ferns and fossil cycads into relation with the living cycads. Especially satisfying is the account of the evolution of the cone, which presents a remarkable series even in living genera. The point of view throughout this section is a conservative one, as is evinced by the treatment of Bennettitales and their relationships.

[1] CHAMBERLAIN, CHARLES J., The living cycads. 8vo, pp. xiv+172. *figs. 91.* The University of Chicago Press. 1919.

Botanists will be glad to have the author's assurance that a more extended and technical account of the living cycads is in preparation. In the present pocket volume of 172 pages the author has done a useful piece of work and has done it well.—M. A. CHRYSLER.

NOTES FOR STUDENTS

Inheritance in Pediastrum.—Practically all of our present knowledge of inheritance in the plant kingdom is based upon work done with flowering plants, regularly involving the sex act. The sex act in flowering plants, furthermore, is peculiarly obscured; we cannot be altogether certain what happens between the time of pollination and seed germination. We think that the program followed is a remarkably regular one, but we feel that frequently irregularities may occur, and we know that sometimes they do. We wish therefore to know what all of these irregularities are, how they affect inheritance, and how they may be induced or controlled artificially. It has long been felt that a study of inheritance in simple plants would be suggestive, for in them many of the complexities surrounding reproduction are stripped away. The sex act takes place "in the open," so that there is more hope of absolute control; some forms even lie "below the level of sex," furnishing unusual material for "pure line" work; and germ plasm seems identical with body plasm. The direct bearing of such a study upon practical genetics may be negligible, but upon theoretical genetics it promises to be profound.

HARPER[2] has been working with cultures of *Pediastrum*, and has developed some very significant ideas. He considers 3 "degrees of directness" of inheritance in *Pediastrum:* (1) direct transmission, as by division of plastids; (2) the more indirect transmission of those adult cell characters (as cell form) which are not visibly present as such in the germ cell; (3) the entirely indirect transmission of the characters of the many-celled organism as a whole (as colony form). The adult cell characters which HARPER observed "do not suggest the working out of influences emanating from elements in the chromosomal organization of the nucleus, but rather the direct expression of the organization of the cell as a whole when it begins to grow," involving specific polarities, surface tension, etc. These cell characters come into expression whether or not the colony is successfully formed. Colony characters, therefore, are dependent upon individual cell characters, rather than the reverse. "If in the swarming period the cell does not achieve its normal position . . . . the maladjustment is never overcome."

Thus the author paints for us two distinct pictures, which should be considered separately. First, the picture of inheritance through specific polarities, etc., of protoplasts as a whole, rather than determiners located on

[2] HARPER, R. A., Organization, reproduction, and inheritance in *Pediastrum*. Proc. Amer. Phil. Soc. **66**:375–439. *pls. 5, 6. figs. 54.* 1918.

chromosomes. This may be a rather general situation among the simpler plants, where germ plasm and body plasm are merged. Whether it is at all applicable to higher plants is questionable. Perhaps the "phylogenetic age" of the latter has brought this difference of body plasm and germ plasm, involving a rigid chromosome mechanism.[3] The other picture is "that the swarming period . . . . is not one of aimless movement . . . . but a definitely directed effort to achieve for each cell a specific relation to its fellows." Successful achievement means normal colonies; otherwise monstrosities result. This situation could apply only to a very limited number of cases, even among the lower plants. Among higher plants a vivid imagination might attempt to apply it to the free nuclear stage in the embryo formation of gymnosperms, or in the organization of the embryo sac of angiosperms. The author, however, does not carry his ideas beyond *Pediastrum*, where they seem quite appropriate and well founded. Similarly careful work upon less peculiar types of algae should yield even more profitable suggestions.—MERLE C. COULTER.

Mendelian inheritance in gametophytes.—One of the most critical tests of the current theoretical mechanism for inheritance lies in the behavior of the gametophyte generation in inheritance. If our Mendelian mechanism is correct, gametophytes should show predictable peculiarities; segregation should take place in the first hybrid generation, and dominance should be out of the question. Such an investigation is not particularly hopeful among angiosperms, owing to the insignificance of the gametophytes. In fact it is a rather general opinion that "the characters which they possess appear to be wholly sporophytic, the factors which they carry functioning only after fertilization."[3] BELLING[4] explains semi-sterility in beans on the basis of the germinal equipment of the gametophytes upon the gametophytes themselves, but this merely involves lethal effects.

More hopeful material is provided by the lower plants, where the gametophyte generation is more prominent and really has characters of its own. TRANSEAU[5] reports hybridization in *Spirogyra*, and it is significant that he can give it a Mendelian interpretation. Unfortunately the work is as yet merely observational rather than experimental. Hybridization was observed taking place in nature between *S. communis* and *S. varians*, *S. varians* and *S. porticalis*. The 3 species involved showed distinguishing characters in the shape and size of the vegetative cells, and the shape and orientation of the zygotes. The auther looked in the immediate vicinity, therefore, for possible hybrids resulting from these crosses which should display new combinations of the parental

[3] EAST, E. M., and PARK, J. B., Studies on self-sterility. I. The behavior of self-sterile plants. Genetics 2:525–609. 1917.

[4] BELLING, JOHN, Lethal factors and sterility. Jour. Heredity 9:161–165. 1918.

[5] TRANSEAU, EDGAR NELSON, Hybrids among species of *Spirogyra*. Amer. Nat. 53:109–119. *figs.* 7. 1919.

characters. He was successful in finding practically all the new combinations that were theoretically possible.

The Mendelian explanation runs as follows: The character of the hybrid zygote itself is maternal, as is to be expected from the cytological behavior during conjugation. The reduction division takes place during the first 2 nuclear divisions of the germinating zygote, but 3 of the resulting nuclei degenerate, so that the cells of the mature filament all have a common ancestor in the fourth nucleus; hence segregation appears in the first hybrid generation, but of course all of the cells of a given filament are alike. Such facts would furnish excellent support for our theoretical mechanism of inheritance, but the author could not be positive as to whether he was dealing with an F_1 or an F_2 generation. It is to be hoped that he will discover how to cultivate this material in the laboratory, and carry the work further under rigid experimental control.—Merle C. Coulter.

Enzyme action.—Van Laer[6] reports some observations on the nature of zymogens, which are claimed to confirm the results of Ford and Guthrie, who had shown that the increase of the amyloclastic activity of papaine with barley meal is not manifested when the infusion is kept in direct contact with the proteo-clastic ferments. The yeast infusions were obtained from yeast prepared according to the Lebedeff method. The addition of papaine to yeast juice destroyed the catalase and zymase. In the state of zymogens, there was shown greater stability and resistance to the factors of inactivation. The hefanal extract of yeast in the presence of antiseptics showed a measurable degree of inverting activity. This inverting agent was amylase. The diastase and papaine had no influence upon the hefanel infusion even after a 24 hours' digestion. Observation is made upon the intensity of autofermentation. After the latter there remains some amylase which is sensitive to papaine. This sensitiveness is expressed in the data as the decrease of the percentage of sugar inverted from 25.6 to 19 when papaine was added. Certain cellulary materials, as soluble or incoagulable protoplasmic products, decreased the activity of sucrase according to the concentration. In the presence of small quantities of these substances the rapidity of hydrolysis of saccharose is hardly modified. Extracts of yeast inactivated by acetone give a notable increase of inverting power when added to solution of papaine or active anylase, the yeast cells in this respect behaving like cellulary bodies. This increase is due on the one hand to the increase of sucrase, and on the other to the decrease of cellulary substance in the digestion products.—A. M. Gurjar.

Buried weed seeds.—Miss Brenchley,[7] on the basis of considerable investigation, makes the following statement concerning the longevity of weed seeds in agricultural soils: "The changes in the proportion of arable and

[6] Van Laer, Henri, Zeits. für Gärungsphysiologie 6:169–175. 1918.

[7] Brenchley, Winifred E., Buried weed seeds. Jour. Agric. Sci. 9:1–31. 1918.

grassland plants derived from buried seeds are so consistent and so regularly associated with the history of the land that one is irresistibly forced to the conclusion that when arable land is grassed over, a certain number of the seeds are able to retain their vitality for very many years. Many of the seeds die within a comparatively short time after burial, and as time goes on the number of living seeds gradually becomes less, although the evidence goes to show that some seeds will survive burial for at least 58 years. Usually most of the older arable seeds survive in the lower depths of soil where the conditions are less variable, whereas the shorter the time that land has been under grass the greater the proportion of arable seeds that are found near the surface. While the stock of arable seeds is diminishing with the lapse of time, the supply of grassland seeds is being augmented by fresh seeds that are ripened by the surface vegetation and are gradually carried down into the soil. Naturally enough, the greater number of these seeds are found in the upper inches of soil, comparatively few penetrating below the eighth inch."

Miss BRENCHLEY fails to note the much earlier and extensive work (1893–94) of PETER, which is very similar to hers in method and conclusion. She also fails to mention the well controlled work of BEAL and of DUVEL on the longevity of buried seeds, which likewise justifies her conclusions.[8]—WM. CROCKER.

Wound callus and bacterial tumor.—Polar difference in wound callus formation has often been observed in stems, and less frequently in root structure. MAGNUS[9] finds that segments of the root of a half long carrot with which he worked produced a wound callus on the morphologically apical face, but not on the basal face. This occurred whether the apical face was oriented upward or downward in the moist chamber. The callus starts at the cambium ring and spreads centripetally. When the apical face is infected with *Bacterium tumefaciens* the callus development is much greater. When the basal face is infected there is a considerable development of tumors on that face, and this acts in a correlative way to inhibit the normal tumor development in the apical face. MAGNUS also worked with a long fodder carrot. While infection in this form increased the callus development on the apical face of the segments tenfold, it induced very little tumor development on the basal face, and accordingly showed little correlative effect in inhibiting the normal development on the apical face.

MAGNUS offers evidence for the view that the tumor inducing organism in plants is not identical with that in man. He also suggests that certain conclusions of BLUMENTHAL and HIRSCHFIELD on the effect of *Diplococcus* in

[8] See CROCKER, WM., Mechanics of dormancy in seeds. Amer. Jour. Bot. 3:99–120. 1916.

[9] MAGNUS, WERNER, Wund-callus und Bakterien-Tumore. Ber. Deutsch. Bot. Gesells. 36:20–29. 1918.

tumor formation in plants may be wrong because they failed to recognize the polar disposition to callus formation. He thinks the studies on tumor formation in plants will finally throw much light on cancer development.—Wm. Crocker.

Effect of illuminating gas on plants.—Wehmer[10] has studied the effect of passing continuous streams of illuminating gas through the soil bearing potted herbaceous as well as 3–7-year-old woody plants. There was a great difference in the amount of injury, according to the stage of development. In the spring the trees were entirely killed in a relatively short time. This is in general the sort of reaction given by the actively growing herbaceous forms at all times. In late summer and early fall the injury is less marked and is shown mainly by leaf fall, while in the dormant period of winter the trees are very resistant. In the cress the embryo in the resting seed and the seedling stage proved very sensitive. Cuttings stood in gas-impregnated water showed, with few exceptions (*Ilex*), seasonal variations in sensitiveness similar to the plants rooted in soils. In spite of this the author thinks that injury to parts above the soil is in part a secondary result of root injury. The injury is due to toxic conditions of the gas and not to mere displacement of oxygen by the gas, as Sorauer has suggested. The toxic constituents increase or decrease with the conditions that lead to an increase or a decrease in the odor-producing materials. A later paper on the toxic constituents is promised. The author seems to have overlooked most of the literature on the effect of illuminating gas on plants.—Wm. Crocker.

Aeration systems of leaves.—Neger[11] has earlier spoken of 2 types of leaves on the basis of the nature of their intercellular systems, heterobaric and homobaric. In a recent article he compares a heterobaric leaf to a house with thousands of rooms lacking communicating doors, and a homobaric leaf to a similar house with communicating doors present and all open. In the first type the intercellular system is divided into many small isolated regions by the smaller veins, with the resulting possibility of different air pressure existing in each; while in the second the whole intercellular system of the leaf is connected and therefore the same pressure exists throughout. Most plants with flat leaves have heterobaric leaves, and the size of the individual chambers varies considerably. In various species of *Quercus* they run from 1/840 to 1/1400 sq. cm., and in *Syringa vulgaris* from 1/8 to 1/10 sq. cm. In the same species shade leaves have larger chambers than sun leaves. The following trees and shrubs have homobaric leaves: *Evonymus japonica*, *Ilex aquifolium*,

[10] Wehmer, C., Leuchtgaswirkung auf Pflanzen. 4. Die Wirkung des Gases auf das Wurzelsystem von Holz-pflanzen; Ursache der Gaswirkung. Ber. Deutsch. Bot. Gesells. 36:140–144. 1918.

[11] Neger, F. W., Die Wegsamkeit der Laubblätter für Gaze. Festschrift zum Ernst Stahl. pp. 152–161. Jena. 1918.

Prunus Laurocerasus, *Hedera Helix*, *Ardisia crispa*, and all needle bearing trees and shrubs. When injured by smoke the homobaric leaves show the injury to the whole leaf due to the gases distributing themselves throughout the whole intercellular system, while the heterobaric leaves show the injury in spots corresponding to individual intercellular chambers.—Wm. Crocker.

Nitrogen fixation by Azotobacter.—Hutchinson,[12] in agreement with Koch, Remy, and others, finds that the nitrogen content of sand or soil may be increased appreciably by the activity of *Azotobacter* when some suitable source of energy is supplied. Sugars proved very effective as an energy source, and distinct gains were obtained with plant residues. In pot cultures the nitrogen gains ran as high as 9 mg. of nitrogen per gram of plant residue added. Even in field cultures additions of sugar increased crop yields 20–54 per cent when conditions were favorable. Hutchinson believes the carbohydrates of plant residues act in a similar way in furthering nitrogen fixation and crop yields. For successful operation of this organism suitable temperature, the presence of phosphates, and a supply of basic material such as calcium carbonate are necessary. Besides these factors, some unknown conditions appear periodically in the soil, interfering with the action of this organism.

The effect of the addition of straw or other crop residues to the soil may be very complex. As important among these effects may be mentioned modification of physical condition of the soil, direct addition of nutrients (in the case of straw, considerable potash, little nitrogen as well as other nutrients), and the indirect addition of nitrogen through furnishing an energy source for *Azotobacter*.—Wm. Crocker.

Fucosan vacuoles.—Hansteen noted that granules, as he called them, accumulate about the chromatophores of Phaeophyceae during carbon assimilation. He thought they were produced by the chromoplasts and were the first visible product of carbon assimilation. On this basis he called them fucosan granules. Kylin[13] has made a rather extensive study of these bodies, the results of which are summarized in the article here reviewed. He finds that these bodies are vacuoles rather than granules, and while they are probably formed by the chromoplast in connection with carbon assimilation, they are not made up in the main of carbon synthate. He thinks he has shown that dextrose is the first carbon synthate of the Phaeophyceae, and that this is condensed to lamanarin. These vacuoles may be the means by which the synthate leaves the plastid, but it is not stored in them. On the contrary, it rapidly diffuses from them into the cytoplasm. He thinks these vacuoles, especially the older ones, are filled with substances resembling tannin, but

[12] Hutchinson, H. B., The influence of plant residues on nitrogen fixation and on losses of nitrate in the soil. Jour. Agric. Sci. 9:92–111. 1918.

[13] Kylin, Herald, Über die Fucosanblasen der Phaeophyceen. Ber. Deutsch. Bot. Gesells. 36:10–19. 1918.

differing from true tannin in some respects. He considers these tannin-like substances as meaningless waste products, which upon oxidation give rise to the brown pigment of this group of plants (phycophaein), which was formerly considered a pigment of the chromatophore.—Wm. Crocker.

Apogamy in Nephrodium.—Steil[14] has uncovered an interesting situation in *Nephrodium hirtipes*, in that the gametophyte never produces archegonia, although antheridia with normal sperms are developed. Secondary gametophytes were induced readily, but only rarely was apospory induced. The embryo begins to develop early in the history of the gametophyte as a vegetative outgrowth, the apical cells of leaf, root, and stem appearing successively, but no foot is formed. No migrations or fusions of nuclei were observed in connection with embryo development. The diploid number of chromosomes is 120–130, and the haploid number (60–65) was observed both in the gametophyte and the apogamous sporophyte. Suggestions are offered as to the origin of such persistent apogamy, the most interesting one being that *N. hirtipes* and other apogamous ferns may be of hybrid origin. The paper is introduced by a very useful summary of our knowledge of apogamy in ferns, beginning with the discovery of tracheids in the gametophyte of *Pteris sulcata* by Leszcyc-Suminski in 1848. The first clear recognition of an apogamous embryo, however, as distinguished from one resulting from fertilization, is credited to Farlow, who in 1874 discovered apogamy in *Pteris cretica albo-lineata*.—J. M. C.

Soil acidity.—Hartwell and Pember[15] find that rye does well on acid soils, while barley is much injured by them. Aqueous extract of acid soil, residue from distillate of the aqueous extract, and the ash of the residue from such distillate affect the two plants much as does the soil itself. Soluble aluminum salts and not the acid in the soils proved to be the source of injury to the barley plants. Addition of acid phosphates, which renders the soil more acid, and lime reduced the solubility of the aluminum salts in acid soils and rendered them non-toxic to barley plants. The authors think these substances often produce beneficial effects in this way rather than by furnishing more available phosphorus or by neutralizing the acid. The reviewer has noticed that the hydrogen ion concentration found in acid soils by the gas chain method is generally only a fraction of the hydrogen ion concentration necessary to reduce the growth rate of plants in water or sand cultures. This work again suggests the complexity of the apparently simple problem of soil acidity.—Wm. Crocker.

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[14] Steil, W. N., A study of apogamy in *Nephrodium hirtipes*. Ann. Botany **33**: 109–132. *pls.* 5–7. 1919.

[15] Hartwell, B. L., and Pember, F. R., The presence of aluminum as a reason for the difference in the effect of the so-called acid soil on barley and rye. Soil Science **6**:259–279. 1918.

Statolith starch.—Miss ZOLLIKOFER[16] finds that the statolith starch of seedling organs is relatively readily removed by periods of illumination followed by periods of darkness. The persistence of the statolith starch is a function of the degree of etiolation. This the writer considers a biological adaptation. By growing seedlings of *Tagetes erecta* and seedlings of other Compositae in light 1–4 days, followed by 3–4 days of darkness, hypocotyls were obtained that bore no statolith starch. These hypocotyls were still growing and capable of phototropic movement, but incapable of geotropic movement. Light rendered them geo-sensitive only after it had produced statolith starch. Working by similar methods the author shows a close relation between the amount of mobile starch and geo-sensitivity in the coleoptile of grasses.—WM. CROCKER.

Fat storage in evergreen leaves.—A number of investigators have stated that there is considerable storage of fats in evergreen leaves during the winter. MEYER[17] finds that the droplets that were supposed by these former workers to be fat droplets are not fat, and that the total volume of these does not rise and fall with winter and summer, but that it increases continuously with the age of the leaf. He speaks of the droplets as "mesophyllsekret," and points out that little is known of their origin and composition. Some of the forms studied were *Ilex aquifolium*, *Taxus baccata*, and *Vinca minor*. The methods used by MEYER, as well as by former workers, are exclusively microchemical. It is evident that these ought to be checked up by quantitative determinations.—WM. CROCKER.

Light and germination.—LEHMANN[18] finds in a germinator at 30° C. 0. 1 second illumination with 730 H.K. is sufficient to cause 50 per cent of the seeds of *Lythrum Salicaria* to germinate within 24 hours, whereas only 6–7 per cent germinate in similar condition in darkness, and not more than 7 per cent after 10 days.—WM. CROCKER.

Osmotic pressure of epiphytes.—HARRIS[19] finds that the epiphytes (Bromeliaceae, Orchidaceae, and Peperomia) of the Jamaican montane forest have 37–60 per cent of the osmotic pressure shown by the herbaceous terrestrial vegetation of the same region, and 28–45 per cent of that of the ligneous terrestrial vegetation.—WM. CROCKER.

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[16] ZOLLIKOFER, CLARA, Über das geotropische Verhalten entstärkter Keimpflanzen und den Abbau der Stärke in Gramineen-koleoptilen. Ber. Deutsch. Bot. Gesells. 36:30–38. 1918.

[17] MEYER, ARTHUR, Die angebliche Fettspeicherung immergrüner Laubblätter. Ber. Deutsch. Bot. Gesells. 36:5–10. 1918.

[18] LEHMANN, ERNST, Über die minimal Belichtungszeit welche die Keimung der Samen von *Lythrum Salicaria*. Ber. Deutsch. Bot. Gesells. 36:157–163. 1918.

[19] HARRIS, J. ARTHUR, On the osmotic concentration of the tissue fluids of phanerogamic epiphytes. Amer. Jour. Bot. 5:490–506. 1918.

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GENERAL INDEX

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